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14. ABSTRACT
A variety of lines of evidence support the possibility that allopregnanolone will be safe and have beneficial effects on disability when administered as a treatment following acute traumatic brain injury (TBI). In this project, a method was developed to manufacture pharmaceutical grade allopregnanolone, which was formulated in intravenous solutions. Regulatory approval was obtained to administer the formulations in the context of a clinical trial in moderate to severe TBI. A placebo controlled, double blind, randomized clinical trial was conducted at the UC Davis Medical Center, a Level 1 trauma center. A dosing regimen based on pharmacokinetic modeling was developed to obtain steady state plasma levels of 50 nM and 150 nM. A total of 13 subjects were enrolled. No treatment related adverse effects were noted. Pharmacokinetic studies conducted in the context of the trial provide a basis for dosing of allopregnanolone in future studies. No safety concerns were raised by the clinical trial. Further research is required to determine if allopregnanolone is efficacious as a treatment for TBI.

15. SUBJECT TERMS
traumatic brain injury, clinical trial, allopregnanolone

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Section I – Introduction

The endogenous neurosteroid allopregnanolone has a variety of biological actions that may confer it with therapeutic activity in the acute treatment of traumatic brain injury (TBI). Of particular relevance to TBI is the ability of allopregnanolone to decrease apoptosis, enhance neurogenesis, and reduce neurodegeneration (Marx et al., 2016). Some or all of these cellular actions may relate, in part, to the action of allopregnanolone as a positive allosteric modulator of $GABA_A$ receptors. Studies in animal models of TBI and other central nervous system injuries including stroke have confirmed the beneficial activity of allopregnanolone as a neuroprotective agent. Specifically, allopregnanolone was found to be efficacious in enhancing neurobehavioral recovery after TBI and decreasing TBI-induced neuronal death (Djebaili et al., 2004; 2005; He et al., 2004ab; Ciriza et al., 2006; Sayeed et al., 2006). These various lines of evidence indicate that treatment with allopregnanolone following TBI in humans may improve long-term outcome by reducing disability and neurological handicap. In this project, a clinical grade intravenous formulation of allopregnanolone was developed. Regulatory approval was obtained to administer the formulation in the context of a clinical trial in moderate to severe TBI to assess its safety and possible efficacy.The trial was conducted at the UC Davis Medical Center, a Level 1 trauma center. Subjects were enrolled and received allopregnanolone treatment or vehicle. Safety, pharmacokinetic and efficacy data were collected.

Section II – Body

The overall objective of this research was to develop a clinical formulation of allopregnanolone for the treatment of TBI and to conduct an initial (Phase 2) clinical study in subjects who had experienced an acute TBI. Several authors have found that greater disability and handicap following TBI are associated with subjective self-reports of poorer outcome. Specifically, individuals with poorer outcome had a higher frequency of depressive symptomatology (McCleary et al., 1998; Wilson et al., 2000). Poor outcome is also associated with reduced mental well-being and problems in neurobehavioral functioning (Wilson et al., 2000). Overall, the improved outcome contemplated by allopregnanolone treatment is expected not only to be associated with improved neurological function but also with an improved subjective sense of satisfaction with life (Wilson et al., 2000). The results of this research are intended to be applicable to the use of allopregnanolone to treat military personnel. Since it is difficult to conduct clinical research in a war zone, this research was conducted in a civilian setting. Nevertheless, the results obtained are applicable to the use of allopregnanolone in a military situation. Such application has the potential to have a positive impact on the function, wellness, and overall quality of life for military Service members affected by severe TBI. Caregivers and families will also be positively impacted since affected Service members with less disability will require less demanding care. The Brain Injury Association of America estimates that the long-term cost of care for a person with severe TBI is $4.1 to 9 million. Such an individual may require 5 to 10 years of rehabilitation and follow-up services. Therefore, in addition to providing improved function, well-being and overall quality of life, the improvement contemplated by allopregnanolone treatment should result in substantial societal cost savings.

In the initial phases of this project, a clinical study protocol was developed, drug manufacturing was completed, an intravenous formulation was developed, an analytical method to assay

allopregnanolone in the drug product was developed, and approvals were obtained from regulatory authorities [(U.S. Food and Drug Administration (FDA) and institutional review board (IRB)].

Clinical Study Protocol. The clinical study protocol was written by the protocol working group. The protocol defined objectives of the study were as follows:

- Primary Objectives: In adults with moderate or severe (Glasgow Coma Score 3–12) traumatic brain injury (TBI): (1) determine the safety of two intravenous (i.v.) doses of allopregnanolone (targeting 50 nM and 150 nM steady-state serum allopregnanolone concentrations) compared to placebo during a 5-day continuous infusion starting within 8 hours after the injury; and (2) to determine the efficacy of intravenous allopregnanolone treatment to improving Glasgow Outcome Score-Extended (GOS-E) at 6 months after injury.
- Secondary Objectives: The clinical benefit of allopregnanolone (Allo) treatment will be further assessed through secondary endpoints including mortality, GOS-E, quality of life, global neurobehavioral function, depression, and late post-traumatic epilepsy 3–12 months after injury.

The following inclusion criteria were defined in the protocol:

- Moderate (post resuscitation GCS 9–12 with abnormal head CT scan) and severe (post resuscitation GCS 3-8) brain injury
- Persons 18 to 65 years old (inclusive) will be eligible for screening for Stage 1; persons 16 to 65 years old (inclusive) will be eligible for screening for Stage 2.
- Closed or blunt traumatic brain injury
- Less than 8 hours from injury to study initiation
- Able to participate for the full term of the study

The following exclusion criteria were defined in the protocol:

- Persons who do not speak English or Spanish
- Life expectancy of less than 24-hrs as determined by the Investigator
- Bilateral non-reactive pupils with GCS 3
- Isolated epidural hematoma
- Hypoxia: pulse oximetry saturation < 90% for 15 or more minutes before enrollment
- Hypotension: systolic blood pressure < 90 mm Hg on 2 or more reliable measurements before enrollment
- Cardiopulmonary arrest prior to randomization
- Spinal cord injury with motor defects
- Pregnancy
- Pre-existing significant disease that could affect outcome
- Prisoner/ward of the state
- Active breast or reproductive organ cancer
- History of thromboembolic events
- Received activated Factor VII before enrollment

- Allergy to progesterone
- Any disease, in the opinion of the Investigator, that is unstable or which could jeopardize the safety of the patient
- Treatment with other investigational drug or procedure in the last 30 days
- Body weight > 120 kg
- Severe renal impairment (creatinine clearance < 50 mg/ml)

The protocol was extensively reviewed by four outside reviewers: Kia Shahlaie, M.D., Ph.D. (University of California, San Francisco), Anne-Marie Guerguerian, M.D. (University of Toronto), Claudia Robertson, M.D. (Baylor College of Medicine), and Paul Vespa, M.D., (University of California, Los Angeles). All reviewer comments were considered by the protocol working group and alterations were made where deemed appropriate. The clinical trial protocol was used in the development of the Pre-IND Package described below and the UC Davis IRB filing.

Pre-IND Meeting Information Package and IND. An extensive Pre-IND briefing document was prepared and submitted with a request for a Type B (Pre-IND) face-to-face meeting with the FDA in accordance with 21 CFR Part 312.82 to discuss the approach to the development of an IND for intravenous allopregnanolone for the treatment of patients with acute TBI. It provides a statement of the purpose and objectives of the meeting and a preliminary proposed agenda. A set of regulatory, clinical pharmacology, and clinical questions were provided. In addition, the Pre-IND Package included an extensive dossier of supporting information and documentation for each of the questions to the FDA. The dossier also included a description of the scientific rationale for the study and an account of the regulatory history mainly focusing on progesterone. The remainder of the Pre-IND Package was a detailed summary of the available scientific literature supporting the safety and potential utility of allopregnanolone in the treatment of TBI. The Pre-IND meeting occurred on May 12, 2011 at the White Oak Campus of the FDA, 10903 New Hampshire Avenue, Silver Spring, Maryland.

Development of Manufacturing Method. The study drug active pharmaceutical ingredient (API) allopregnanolone had not previously been manufactured in the quantities and to the purity specifications required. Moreover, the requirement that the manufacturing be conducted according to cGMP markedly increased the difficulty.

The synthetic pathway as originally conceived is as show in Fig. 1 below.

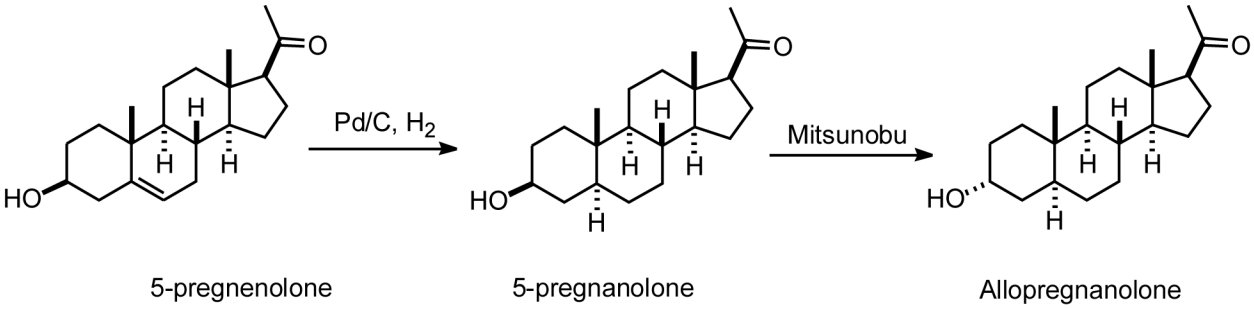


Fig. 1. Synthetic scheme for preparation of allopregnanolone (3α-hydroxy-5α-pregnan-20-one).

This scheme resulted in contamination of the final product with the byproduct triphenylphosphine oxide. This was present at a concentration of several percent and could not be removed by repeated crystallization. An alternative approach resulted in a product of adequate purity. Specific details for each of the reaction steps are as follows:

Step 1. 5-Pregnenolone to 5-Pregnanolone

It was found that this step could be accomplished using a Paar apparatus at 30-40 psi of hydrogen with a dry 10% palladium on carbon catalyst. The yield was almost quantitative and less than 1% of byproducts were formed. At lower pressures hydrogenation was much slower. However, for safety reasons a wet catalyst was substituted. Several problems were encountered in laboratory scale synthetic runs and a larger qualification runs. The solution temperature would upon adding hydrogen rise to 50-60 °C and this would result in the formation of substantial amounts of impurities. Lower hydrogen pressures and shorter reaction times did not solve this problem. Eventually, it was determined that by careful cooling these issues could be reduced and that pure material could be produced in good yield. However, there seemed to be substantial batch-to-batch variability in the hydrogenation conditions, probably due to slight changes in catalyst activity. For example, an initial 200 g qualification run proceeded slowly. Accordingly, it was tried again heating from only 20 to 25 °C. This produced a temperature spike to about 50 °C, which resulted in product that contained about 15% of various unidentified diastereomeric impurities. Accordingly great care was taken in the final cGMP run to cool the reaction and avoid temperature spiking.

There were also considerable experimentation required to identify the appropriate solvent for the reaction. Due to the relatively modest solubility of the starting material in ethanol, it was considered desirable to investigate the use of other solvents and cosolvents. These included (a) 1:1:0.2 ethanol/tetrahydrofuran/acetic acid, (b) 1:1 ethanol/2-methyl tetrahydrofurna, (c) 20% (v/v) dichloromethane in ethanol, (d) 20% methanol in dichloromethane. Of these system (d) gave complete conversion and was selected for the final GMP run. Filtration of the catalyst through celite with the use of glass fiber filter paper was found to be sufficient to reduce levels of palladium and other metals in the final product to an acceptable (ppm) range. Some issues were found with regard to lowered yields due to retention of some material on the celite cake, but this could be remedied by washing with excess hot solvent.

Step 2. Mitsunobu Reaction

It was initially found that using triphenylphosphine the Mitsunobu reaction proceeded well but residual triphenylphosphine oxide was formed which was present at a level of about 5% in the final product. This was found to be impossible to remove by repeated recrystallization. Thus, the use of triphenylphosphine in the Mitsunobu reaction was a potential concern. Initially some effort was made to substitute tributylphosphine for triphenylphosphine oxide. However, this effort was not successful.

As other phosphines are substantially more expensive it was not feasible to adopt an alternative phosphine. It was therefore necessary to develop a means of removing the triphenylphopshine oxide by some appropriate process. A suggestion by Dr. Robert Purdy (San Diego Veterans

Administration Hospital) was to make the 2,4-dinitrobenzoyl ester of pregnanolone, which was more readily separated in his experience from the triphenylphosphine oxide. This concept was modified to use *p*-nitrobenzoic acid in the Mitsunobu reaction instead of trifluoroacetic acid as the donor molecule. This procedure resulted in excellent yields of the *p*-nitrobenzoyl ester, which could be freed almost completely from the triphenylphosphine oxide by a single recrystallization.

The revised synthetic scheme is shown in Fig. 2.

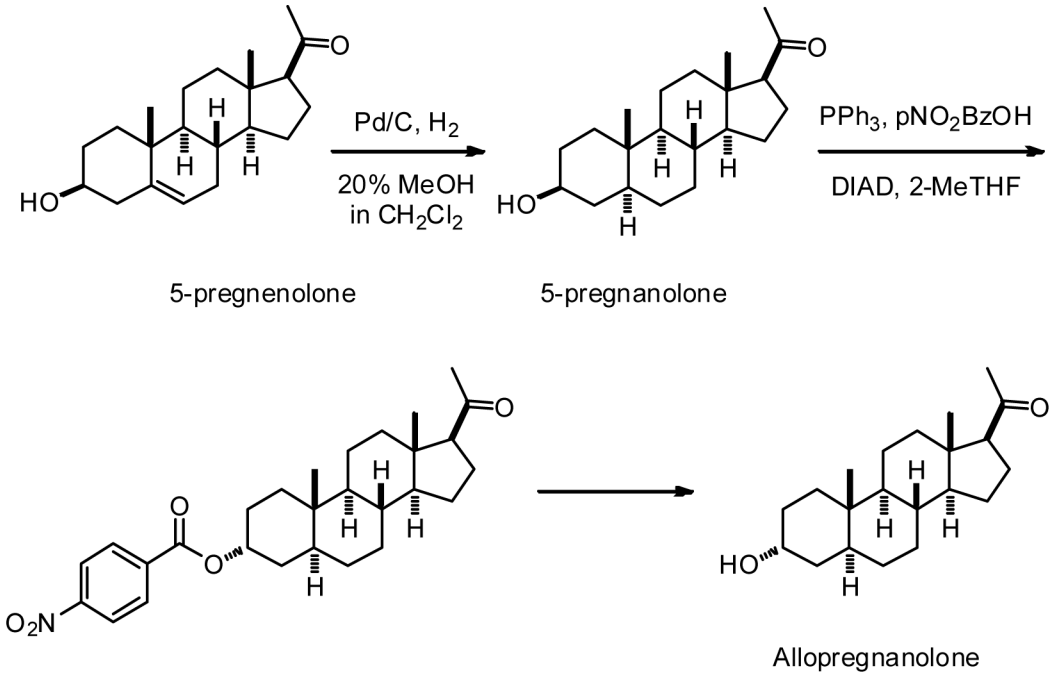


Fig. 2. Revised synthetic scheme with *p*-nitrobenzoic acid (pNO2BzOH) in the Mitsunobu reaction.

Step 3. Hydrolysis

Multiple attempts using different reaction conditions were tried to hydrolyze the *p*-nitribenzoic acid derivative to the final product allopregnanolone. Reaction 388-130 utilized 1 N NaOH in methanol and tetrahydrofuran at a temperature of 35 °C. This resulted in a 40% isolated yield for the main lot with the product displaying 93% HPLC purity. The main impurity was *p*-nitrobenzoic acid. The low yield was due to the fact that ethyl acetate was used as the crystallization and wash solvent. The product was found to be more soluble in ethyl acetate than originally envisioned and this resulted in the low yield.

Reaction 388-131 was run on 15 g scale and was complete after about 5 hours. The work-up of the reaction was split and half of the organic layer was retained. A modified work-up compared to reaction 388-130 was used on the other half in which the organic layer was washed with twice as much NaOH as in 388-130, and then washed twice with water. The organic layer was concentrated to 10 volumes, and 10 volumes of heptane were charged over 10 minutes while the slurry was stirring. The slurry was then concentrated to 10 volumes, stirred at room temperature overnight, and then cooled to 0 °C for 4 hours. After filtration and heptane washing, the product

was dried at 40 °C overnight. Residual solvent analysis of the slurry supernatant prior to filtration showed that it contained 28% ethyl acetate. Based on these experiments, the optimal conditions were determined to be as follow: material was redissolved in ethyl acetate/methanol, washed twice with 1 N NaOH, twice with water, and concentration until ~7 volumes of ethyl acetate remained. The slurry was then heated to fully dissolve the material and heptane was added to precipitate allopregnanolone. After isolation and drying, a 59.2% recovery was obtained.

The purity of the isolated material is indicated by the results of a typical HPLC run illustrated in Figs. 3 and 4. The impurities noted will be identified using chemical methods and also by running known compounds on HPLC.

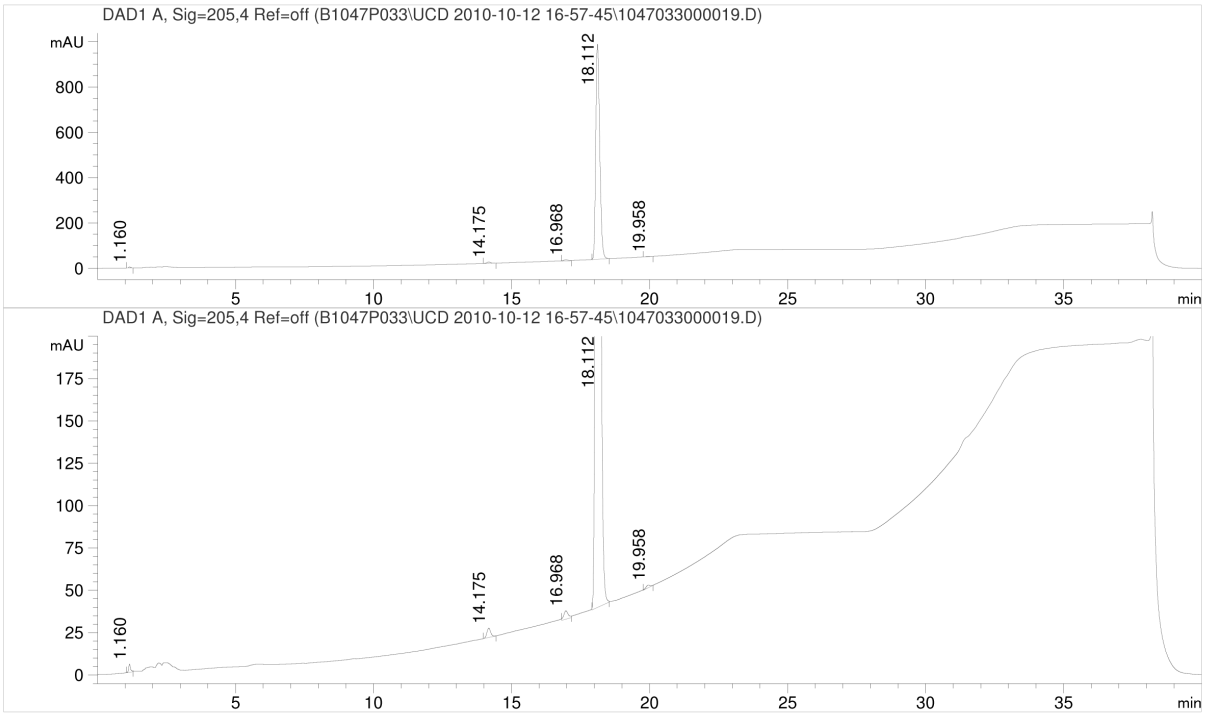


Fig. 3. Representative HPLC analysis of qualification run material late in the development of the synthetic route. Allopregnanolone is at retention time 18.112. Four impurities are noted.

#	Meas. R	Height	Width	Area	Area %
1	1.160	4.822	0.068	22.426	0.232
2	14.175	5.558	0.147	52.750	0.545
3	16.968	4.963	0.190	56.599	0.585
4	18.112	949.925	0.167	9525.206	98.461
5	19.958	1.563	0.183	17.153	0.177

Fig. 4. Quantification of the components from the HPLC trace shown in Fig. 3. Four unidentified impurities were present, representing 1.539%. These components were substantially reduced in the final production run to less than 0.3% each. The levels meet FDA standards as less than 1 mg is expected to be administered daily.

Step 4. Final Recrystallization

Unfortunately, in later runs small amounts of impurities, presumably formed in the Mitsunobu reaction, begin to appear in the final product. Therefore renewed attempts were made at recrystallization to remove these impurities. It was determined that a recrystallization of allopregnanolone from 10 L/kg of ethanol and 7 L/kg of water was able to increase the product purity to well within the specification of 98% purity. Manufacturing process development was therefore deemed successful. The API specification in shown in Appendix I and the certificate of analysis for the process development run is shown in Appendix IIA.

GMP API Manufacturing. Upon completion of the major steps in the development of the manufacturing process, it was possible to begin cGMP manufacturing of the allopregnanolone API. Manufacturing was performed using 19.8 kg of 5-pregnenolone as starting material. The 5-pregnenolone was dissolved in 20% methanol/dichloromethane, charged to 0.99 kg of 10% palladium on carbon, and allowed to hydrogenate under 20 PSI at 20 ± 5 °C. At 30 hours the reaction was sampled and deemed complete with only 0.2% 5-pregnenolone remaining relative to 5-pregnanolone. The 10% palladium on carbon was filtered off as the product containing solution was transferred into a 200-gallon reactor. A solvent switch to 2-methyltetrahydrofuran (2-Me-THF) was performed. Subsequently, 13.5 kg of 4-nitrobenzoic acid and 20.4 kg of triphenylphosphine were charged to the reactor. While operators were cooling the slurry to 0 ± 5 °C, solids settled to the bottom due to inadequate stirring. To aid in better agitation, 43.0 kg of 2-methyltetrahydrofuran was added to the reactor. Once sufficient agitation was reached and the temperature was within range, 18.2 kg of DIAD was slowly added to the reactor. The reaction mixture was warmed to 20 ± 5 °C and the Mitsunobu reaction began.

The Mitsunobu reaction stirred at 20 ± 5 °C for 15 hours and 50 minutes, until 0.1% 5-pregnanolone remained relative to 4-NO_2Bz-allopregnanolone. A solvent switch was performed from 2-Me-THF to IPA until residual 2-Me-THF remaining was < 0.1%. The slurry was filtered, and the filter cake was washed twice with isopropyl alcohol (IPA). 2-propanol was used for the remainder of the process.

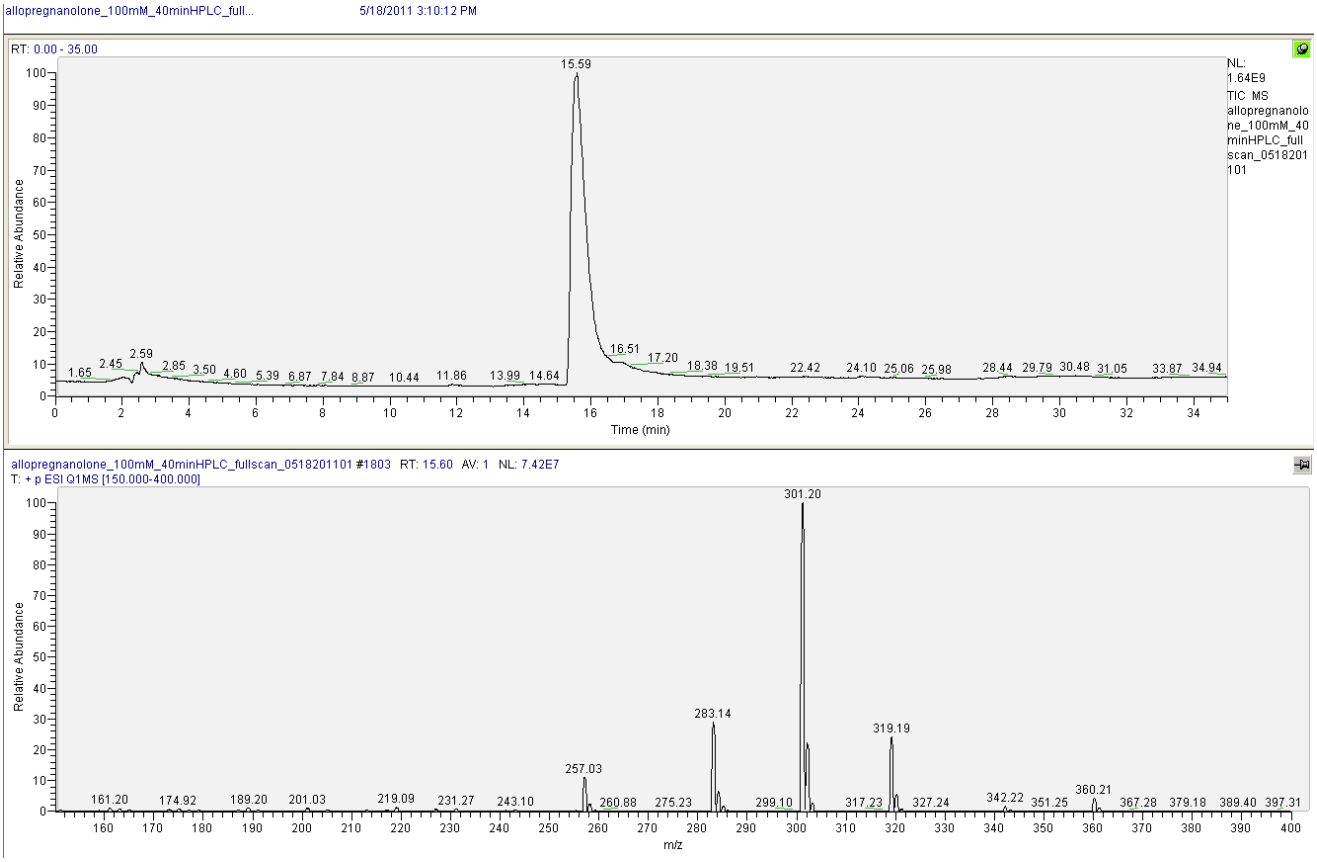
The 4-NO_2Bz-allopregnanolone was isolated and dried for 66 hours and 28 minutes, and was deemed 1% dry by thermogravimetric analysis. The product was reslurried for 6 hours and 15 minutes, washed with IPA, isolated, and allowed to dry for 18 hours and 8 minutes. It was deemed 1% dry by TGA. A sample was analyzed by HPLC and the purity was 100% (rounded from 99.7%) with <0.01% triphenylphosphine oxide. The product was discharged with a net weight of 24.9 kg representing an 85% yield going into step 3.

The product was slurried in THF and MeOH with 1M NaOH added over 75 minutes, maintaining internal temperature below room temperature. The hydrolysis reaction was allowed to stir for 8 hours and 47 minutes until 0.2% (uncorrected) 4-NO_2Bz-allopregnanolone remained relative to allopregnanolone. The aqueous workup was performed very slowly due to large emulsions that

were observed. The organics were polish filtered once a pH of 7 of the water washes was reached, and the distillations were performed until 0.6% residual THF remained. The recrystallization was performed and allopregnanolone was dried.

API Purity. Following the successful completion of the GMP synthesis of the API, the API was stored under controlled conditions in the UC Davis Good Manufacturing Practice Laboratory. The API was 99.9% pure by weight. A series of analyses were conducted to assure the purity, integrity and stability of the API. In addition, attempts were made to identify the nature of the minor impurities that had been detected by HPLC. Reference standards of 5 possible impurities were provided by Dr. Robert Purdy, University of California, San Diego. These were 3β-hydroxy-5αa-pregnan-20-one (B), 3α-hydroxy-5β-pregnan-20-one (E), 3β-hydroxy-5β-pregnan-20-one (F), 5α-pregnane-3α,20α-diol (C), 5β-pregnane-3α,20α-diol (G). LC/MS analysis was performed with a Waters® ACQUITY UPLC stack (Waters Corporation, Milford, MA) equipped with a Hibar® RT 250-4 LiChrosorb® RP-18 (5 μm) column (Merck Chemicals, Gibbstown, NJ) coupled to an electrospray ionization TSQ Quantum Access MAX mass spectrometer (Thermo Fisher Scientific, Waltham, MA). The mobile phase consisted of acetonitrile and water, each with 0.2% formic acid. The 40-minute chromatography run had a flow rate of 1.0 mL/min, and a gradient starting from 40/60 (acetonitrile/water) to 90/10 in 20 min, then to 100/0 over 10 min, and finally back to 40/60 at 36 min. With the column maintained at room temperature (approximately 25 °C), allopregnanolone showed a retention time of 15.59 min. The peak was detected using electrospray ionization mass spectrometry (spray voltage 4000 V; vaporizer temperature 450 °C; sheath gas pressure 60 arbitrary units; ion sweep gas 2 arbitrary units; auxiliary gas pressure 35 arbitrary units; capillary temperature, 300 °C; tube lens offset 79 V; positive ion mode). Allopregnanolone was detected and quantified by its base peak at a mass of 301 [= break-down product from 319 (MH+)].

Figure 5. LC/MS Analysis of GMP Allopregnanolone.



Since allopregnanolone and the 5 impurity samples do not ionize well with the ESI source of the ThermoFisher TSQ Quantum Access MAX mass spectrometer, 100 mM allopregnanolone were used for the analysis in order to obtain the required sensitivity to detect impurities at the 0.1-1% level. Allopregnanolone was first run in Full Scan mode, which in addition to allopregnanolone at 15.59 min, showed additional small peaks as shown in the chromatogram in Figure 1. In order to increase sensitivity SRM (selective reaction monitoring) chromatograms were collected from the 5 impurity sample provided as references and from allopregnanolone at a concentration of 10 and 100 mM. In the SRM mode (monitoring break-down from 319 [M+H] to 301), a peak at 16.90 RT was detected right next to allopregnanolone. By comparing the retention time and major ions from this impurity with the provided standards, this impurity could be identified as 3β-hydroxy-5α-pregnan-20-one (B).

Monitoring the breakdown from 301 [M+H] to 285 in the SRM mode, we could detect additional peaks at 14.85 RT and 24.66 RT. The peak at 14.85 most likely results from either 5α-pregnane-3α,20α-diol (C, RT 14.93) or its enantiomer 5β-pregnane-3α,20α-diol (G, RT 14.23). The impurities found at 11.86 RT and 24.66 RT could not be identified since no impurity standards were available. However, based on the major ions present in both peaks both peaks are likely from other steroid diols. It was concluded that the API contains 4 minor impurities (3β-hydroxy-5α-pregnan-20-one, 5β- or 5α-pregnane-3α,20α-diol, and two steroid diols). Together these impurities constitute less than 1%.

An additional analysis of the same batch was made using an Avance 800 (Bruker) 800 MHz NMR at the University of California, Davis NMR Facility MS1-D. The studies were performed by Dr. Jerry Dallas (NMR facility manager). Quantitative ^{1}H and ^{13}C-NMR were conducted. No additional signals could be identified in the ^{13}C or any impurities greater 2% in the ^{1}H-NMR. The concentrations of the samples were 3 mg/ml (~10 mM), 10 mg/ml (~30 mM) and 40 mg/ml (~125 mM). The ^{1}H-NMR spectrum matched the expected spectrum for allopregnanolone. The ppm values were identical with the ones found in reference spectra. The integrals showed the presence of 34 protons. No impurities greater than 2% (integral: ≤0.02) could be detected.

The ^{13}C-NMR spectrum matched the expected spectrum for allopregnanolone. The ppm values were identical with the ones found in reference spectra. The quantitative ^{13}C experiment showed 21 signals with the integrals in the range of 0.73 to 1.2 representing 21 carbons as expected for allopregnanolone. (The integral values are in the range of expected experimental error). No additional signals (corresponding to impurities) of lower or higher intensity could be detected.

The results of the further characterization of the API as described above were included in the CMC section of the IND submitted for FDA review. The certificate of analysis for the GMP grade material is shown in Appendix IIB.

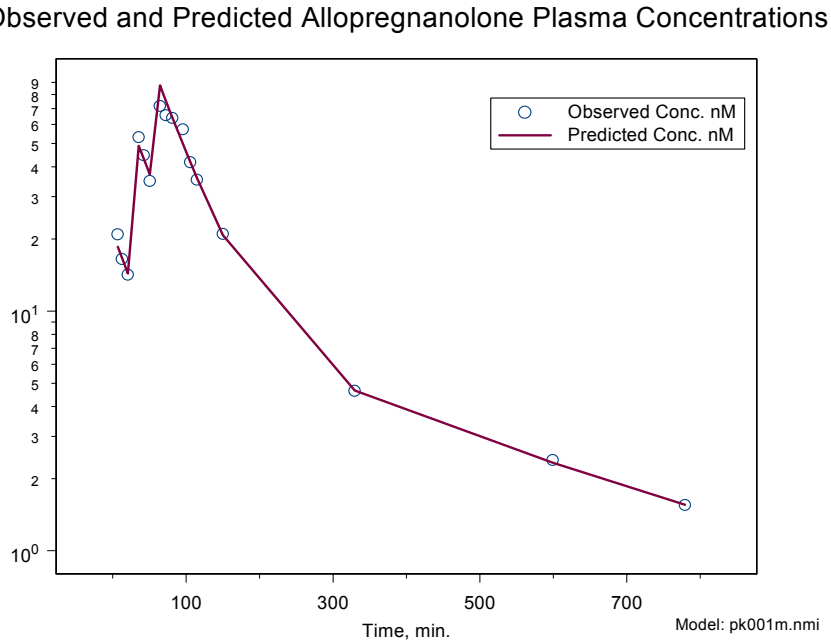
Analytical Method Development. An HPLC separation method for allopregnanolone was developed using an Agilent LiChrospher 100 reverse phase (C18) 250 × 4 mm, 5 μ column. The mobile phase was acetonitrile:methanol (325:25). The separation is run at room temperature and detection utilized UV at 205 nm. Total run time is 30 min and equilibration time is 4 min. This assay has been used to verify the allopregnanolone in the drug product formulation.

Allopregnanolone Pharmacokinetic Modeling and Simulation. The study planned to target a blood concentration of allopregnanolone expected to have been achieved with progesterone in previous TBI trials, which was determined to be approximately 50 nM. To avoid underdosing, provisions were made to allow for a greater target plasma concentration. The FDA indicated that they would require additional supportive safety data (i.e., nonclinical safety data) if doses were to be administered that produce plasma concentration that are substantially above 157 nM (or levels established during pregnancy). Therefore, the project team decided to create formulations and dosing schemes that would target two target steady-state blood concentrations, 50 nM and 150 nM. This required the development of three product forms: Product Form P: *placebo* (0 mg/mL); Product Form L: *50 nM target* (0.5 mg/mL) and Product Form H: *150 nM target* (1.5 mg/mL).

Dosing was determined based on the body weight of the subject. Each subject received a volume of product that is the same for each product forms. Therefore, irrespective of which group the subject was randomized, the volume and quantity of excipients administered are identical. In order to determine the dosing required to achieve the target allopregnanolone levels, modeling was conducted based upon the data reported in the study by Timby et al. (2006). In this study, increasing intravenous doses (15, 30 and 45 μg/kg) of allopregnanolone were administered to healthy female volunteers. The data from this study were modeled using a two compartment linear pharmacokinetic model. Concentrations were reported in units of nM and doses were converted to nM/kg prior to modeling (using a molecular weight for allopregnanolone of

318.49). The analysis was conducted using NONMEM v7.2 (ICON plc) and TIBCO Spotfire S+® 8.2 (TIBCO Software Inc). Figure 6 depicts observed and predicted plasma concentrations for the proposed model.

Figure 6. Mean Observed and Predicted Plasma Allopregnanolone Concentrations in 10 healthy Female Volunteers Following Increasing Intravenous Doses of Allopregnanolone Based on Timby et al. (2006)



Pharmacokinetic parameter estimates for the model are summarized in Table 1.

Table 1. Pharmacokinetic Parameters for Allopregnanolone

Parameter	Estimate	SEE	%CV
CL, L/kg/min	0.0297	0.000473	1.59
CL, mL/kg/min	29.7		
CL, L/kg/hr	1.782		
Q, L/kg/min	0.0126	0.000645	5.12
Q, L/kg/hr	0.756		
V1, L/kg	2.25	0.0972	4.32
V2, L/kg	3.73	0.114	3.06
Vdss, L/kg	5.98		
Residual Error % CV	7.97		

CL is clearance, Q is inter-compartmental clearance; V1 and V2 are the central and peripheral compartment volumes of distribution, and Vdss is the steady state volume of distribution.

The clearance estimate of 29.7 ml/min/kg is similar to the value reported by Timby et al. (32.6 ± 5.8 ml/min/kg). The parameters in Table 2 were used to simulate loading and maintenance intravenous infusion regimens, which targeted steady state allopregnanolone plasma concentrations of 50 and 150 nM. For these simulations it was assumed that drug clearance

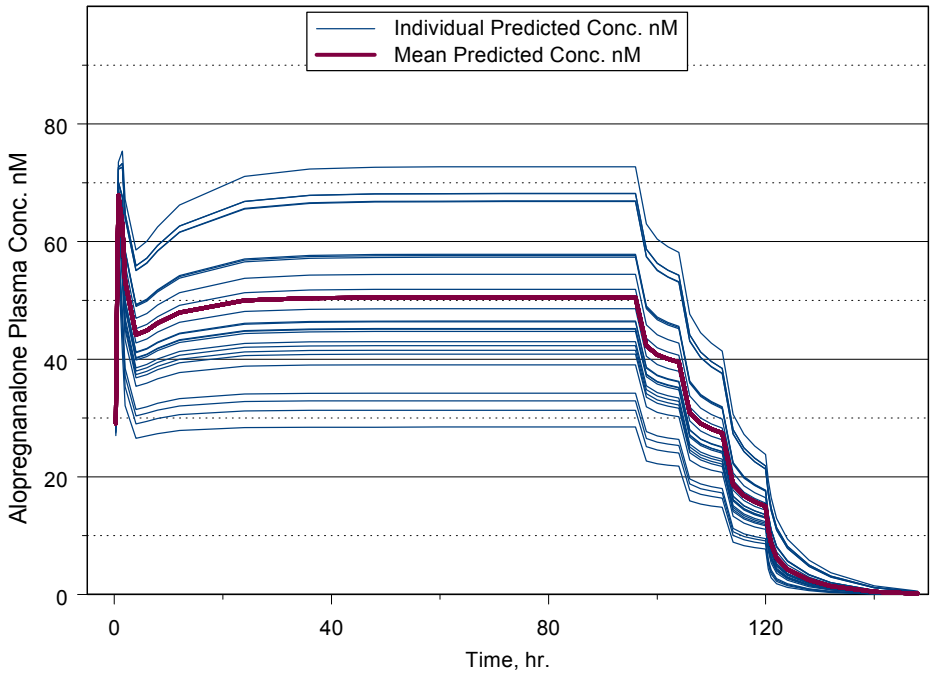
would have a 25% CV for inter-individual variation in the parameter value, reflecting the between subject variability in this parameter reported for CL by Timby et al. (2006).

The simulation provided the dosing regimen given in Table 2 for the group of patients in the 50 nM steady state plasma allopregnanolone target group. This dosing regimen is predicted to produce the plasma allopregnanolone time course as shown in Figure 7.

Table 2. Dosing Regimen for 50 nM Steady State Plasma Allopregnanolone Concentration

Target Css = 50 nM	Infusion Rate	Start Time, hr	Stop Time, hr
Loading nM/kg/hr	300	0	1
Loading μg/kg/hr	95.5		
Maintenance nM/kg/hr	90	1	96
Maintenance μg/kg/hr	28.7		
75% Maintenance nM/kg/hr	67.5	96	104
75% Maintenance μg/kg/hr	21.5		
50% Maintenance nM/kg/hr	45	104	112
50% Maintenance μg/kg/hr	14.3		
25% Maintenance nM/kg/hr	22.5	112	120
25% Maintenance μg/kg/hr	7.2		

Figure 7. Time-course of Allopregnanolone in 25 Simulated Individuals Receiving the Infusion Regimen Described in Table 2

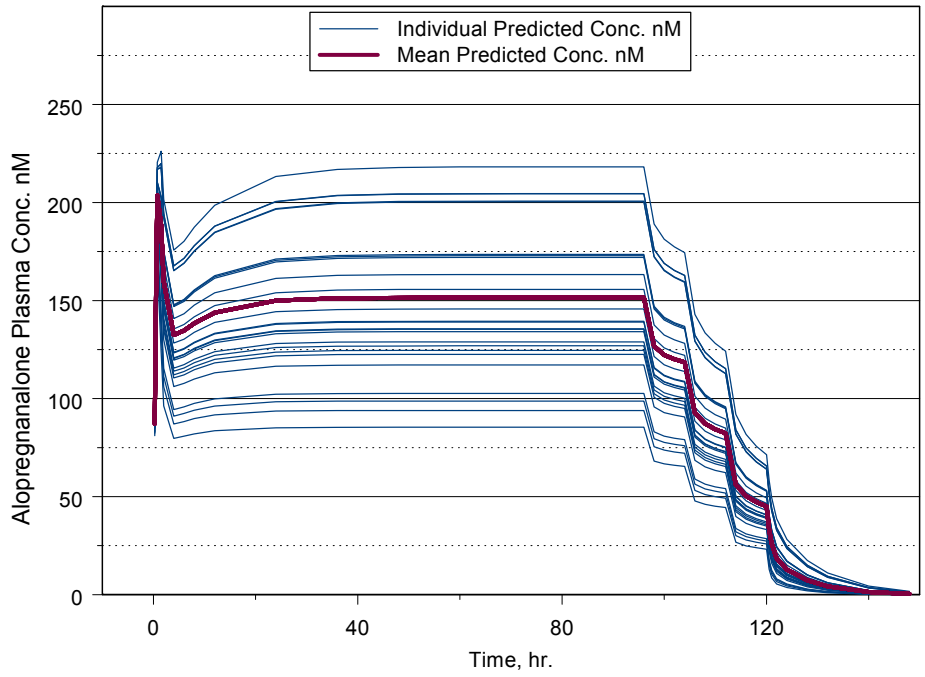


The simulation provided the dosing regimen given in Table 3 for the group of patients in the 150 nM steady state plasma allopregnanolone target group. This dosing regimen is predicted to produce the plasma allopregnanolone time course as shown in Figure 8.

Table 3. Dosing regimen for 150 nM Steady State Plasma Allopregnanolone Concentration

Target Css = 150 nM	Infusion Rate	Start Time, hr	Stop Time, hr
Loading nM/kg/hr	900	0	1
Loading μg/kg/hr	286.6		
Maintenance nM/kg/hr	270	1	96
Maintenance μg/kg/hr	86.0		
75% Maintenance nM/kg/hr	202.5	96	104
75% Maintenance μg/kg/hr	64.5		
50% Maintenance nM/kg/hr	135	104	112
50% Maintenance μg/kg/hr	43.0		
25% Maintenance nM/kg/hr	67.5	112	120
25% Maintenance μg/kg/hr	21.5		

Figure 8. Time-course of Allopregnanolone in 25 Simulated Individuals Receiving the Infusion Regimen Described in Table 3



The simulations demonstrate that a 1 hour loading infusion of the amount calculated as the product of the target steady state plasma concentration (nM) times the total volume of distribution (V_{dss} = 5.98 L/kg) produces a modest initial overshoot of the target steady state concentration. This is followed by a 10 to 24 hour period where concentrations initially fall below target but eventually reach steady state. This pattern is typical of a compound with multi-compartment pharmacokinetics. The approach chosen is a compromise that minimizes the initial over-shoot and the subsequent undershoot.

There is uncertainty regarding the CL of allopregnanolone that will be encountered in a TBI patient population. There may well be gender differences, and it is possible that CL will be altered by the disease state, as it is for progesterone. Therefore, in the trial design, an opportunity

was provided for dose adjustment if the predefined target plasma levels were not achieved. Such dose adjustment was not required.

Formulation. Allopregnanolone is a highly hydrophobic substance that does not dissolve to any appreciable extent in aqueous solution as is required for intravenous administration. Various formulation strategies were attempted, including the formulation of 5 mg/ml allopregnanolone in a 25% human albumin solution. However, concerns arose as to the safety of human albumin. Albumin is derived from human plasma and as such may contain infectious agents, such as viruses, that can cause disease. Other issues include the risk of developing circulatory overload and pulmonary edema with rapid administration. Adverse reactions such as nausea, fever, chills or urticaria can occur although they are rare. Apart from the safety issues, the albumin formulation is less than ideal for deployment into the field, such as in a combat situation. Therefore, an alternative formulation was sought. Cyclodextrins, such as hydroxypropyl-β-cyclodextrin (HPBCD) or β-cyclodextrin sulfobutyl ethers, sodium salts (SBEBCD), can be used to solubilize hydrophobic small molecules. They are present in FDA-approved products for intravenous administration and have a good safety record. Experimentation allowed us to determine an acceptable concentration of cyclodextrin (6%) that was able to solubilize allopregnanolone in a solution with acceptable osmolality, pH and particulates to meet USP specifications for intravenous drug products.

Guidance was obtained from the FDA that there was no concern with the use of a cyclodextrin. Therefore, having completed pharmacokinetic modeling and the definition of the dosing regimen, it was possible to define the optimal product specifications. Concentrations of API in the product forms were constrained by the flow rates that can be delivered by standard intravenous infusion pump systems (minimum flow rate, 0.5 mL/h). It was determined that the product form should be hyperosmolar for use in brain injured patients. To minimize the risk of renal toxicity, attempts were made to minimize the concentration of cyclodextrin. These various considerations led to the development of P, L and H product forms containing, respectively, 0, 0.5 and 1.5 mg/mL allopregnanolone in 6% cyclodextrin (either HPBCD or SBEBCD) in 0.9% sodium chloride injection, USP.

The product forms were manufactured in 4-fold stock solutions and frozen at –20 º. Stability testing was accomplished on the frozen concentrate used as an intermediate in manufacturing and the final product forms produced by diluting the stock solutions with 0.9% sodium chloride injection, USP.

The certificates of analyses for the final product forms are given in Appendix III. The certificates of analyses for the 4-fold concentrates are given in Appendix IV. The product label is shown in Appendix V.

Dosing. Each enrolled subject received one of the following product forms administered according to the dosing regimen shown in the table below: P – placebo, L – allopregnanolone to target 50 nM plasma concentration, or H – allopregnanolone to target 150 nM plasma concentration. Product P consists of 6% cyclodextrin (HPBCD or SBEBCD) in 0.9% sodium chloride injection, USP. Product L consists of 0.5 mg/mL allopregnanolone in 6% cyclodextrin in 0.9% sodium chloride injection, USP. Product H consists of 1.5-mg/mL allopregnanolone in

6% cyclodextrin in 0.9% sodium chloride injection, USP. Dosing is determined based on total (actual) body weight. There are 8 infusion phases. A separate bag labeled according to the "bag designation" given in Table 4 below is administered for each of the 8 phases. The volume of product in the bag is customized for the subject based upon the dose to be delivered. Intravenous bags of the standard product forms were manufactured in the UC Davis Good Manufacturing Practice Laboratory. The bags were delivered for temporary storage under controlled conditions in the Investigational Drug Service (IDS) pharmacy. Customization of the volume in the bags was carried out by IDS as required by each enrolled subject before use. The product in the bag was administered over the period of time in hours as indicated in Table 4. The infusion rate was customized for each subject according to body weight with the intention of delivering the entire contents of the bag over the specified infusion time.

Table 4. Three Dosing Regimens for Allopregnanolone

Day	Bag Designation (Phase)	Time (hr)	Product Form P (allopregnanolone μg/kg/hr)	Product Form L (allopregnanolone μg/kg/hr)	Product Form H (allopregnanolone μg/kg/hr)
1	D1-1 (Load)	1	0	95.5	286.6
1	D1-2 (Maintenance)	23	0	28.7	86.0
2	D2-1 (Maintenance)	24	0	28.7	86.0
3	D3-1 (Maintenance)	24	0	28.7	86.0
4	D4-1 (Maintenance)	24	0	28.7	86.0
5	D5-1 (Taper 75%)	8	0	21.5	64.5
5	D5-2 (Taper 50%)	8	0	14.3	43.0
5	D5-3 (Taper 25%)	8	0	7.2	21.5

Assuming a 70 kg subject, Table 5 below gives the amounts of allopregnanolone (in mg) delivered during each phase. In this hypothetical situation, the total amounts of allopregnanolone that would be administered during the course of the study (8 phases) per 70 kg subject for product form P, product form L and product form H are 0 mg, 221.6 mg, and 664.2 mg, respectively.

Table 5. Total Allopregnanolone Delivered in Three Dosing Regimens for 70 kg Subject

Day	Bag Designation (Phase)	Time (hr)	Product Form P (allopregnanolone mg)	Product Form L (allopregnanolone mg)	Product Form H (allopregnanolone mg)
1	D1-1 (Load)	1	0	6.7	20.1
1	D1-2 (Maintenance)	23	0	46.2	138.5
2	D2-1 (Maintenance)	24	0	48.2	144.5
3	D3-1 (Maintenance)	24	0	48.2	144.5
4	D4-1 (Maintenance)	24	0	48.2	144.5
5	D5-1 (Taper 75%)	8	0	12.0	36.1
5	D5-2 (Taper 50%)	8	0	8.0	24.1
5	D5-3 (Taper 25%)	8	0	4.0	12.0
	TOTAL	**120**	**0**	**221.6**	**664.2**

Again assuming a 70 kg subject, for all 3 product forms, the amounts of HPBCD that would be administered during each phase are as shown in the Table 6 below. The total amount of cyclodextrin that would be administered during the 8 phases (5 days) is 26.6 g.

Table 6. Cyclodextrin Delivered with All Three Dosing Regimens

Day	Bag Designation (Phase)	Time (hr)	Cyclodextrin (g)
1	D1-1 (Load)	1	0.80
1	D1-2 (Maintenance)	23	5.54
2	D2-1 (Maintenance)	24	5.78
3	D3-1 (Maintenance)	24	5.78
4	D4-1 (Maintenance)	24	5.78
5	D5-1 (Taper 75%)	8	1.44
5	D5-2 (Taper 50%)	8	0.96
5	D5-3 (Taper 25%)	8	0.48
	TOTAL	**120**	**26.6**

Table 7 below gives the volume of product to be administered on a mL/kg basis and in mL for a hypothetical 70 kg subject over the indicated time period. The infusion rate is adjusted so that the volume is administered uniformly over the phase period. The infusion rate is specified on the product label and entered by the pharmacist into the patient order record.

Table 7. Volume of Product by Body Weight and Total Infusion Volume and Infusion Rate for 70 kg Subject

Day	Bag Designation (Phase)	Time (hr)	Product volume (mL/kg)	Product volume for 70 kg subject (mL)	Infusion rate for 70 kg subject (mL/hr)
1	D1-1 (Load)	1	0.191	13.37	13.37
1	D1-2 (Maintenance)	23	1.319	92.31	4.01
2	D2-1 (Maintenance)	24	1.376	96.32	4.01
3	D3-1 (Maintenance)	24	1.376	96.32	4.01
4	D4-1 (Maintenance)	24	1.376	96.32	4.01
5	D5-1 (Taper 75%)	8	0.344	24.08	3.01
5	D5-2 (Taper 50%)	8	0.229	16.05	2.01
5	D5-3 (Taper 25%)	8	0.115	8.03	1.00
	TOTAL	**120**		**166.06**	

A dedicated infusion line was used for the administration of the final product into a peripheral vein or a central vein, if a peripheral vein was unavailable. No concomitant medications were added to the same bag or administered through the same line. The infusion line/catheter was flushed with 0.9% sodium chloride for infusion, USP at the end of the infusion to insure delivery of the entire dose of product. Other medications may be given after flushing or another lumen may be used in the case of a multilumen catheter.

Pre-IND Meeting. In preparation for IND submission, the project team participated in a Pre-IND meeting with the Division of Neurology Drug Products, FDA to address several questions regarding aspects of the IND related to regulatory, CMC, nonclinical, clinical pharmacology, and clinical issues. The Pre-IND meeting occurred on May 12, 2011. Advice from the FDA was obtained concerning dosing. The FDA reinforced the need to assess the pharmacokinetic properties of allopregnanolone in patients with TBI. They confirmed our plan to measure allopregnanolone at 17 time points for each subject following the onset of dosing and instructed us to make additional measurements; our revised protocol will include 20 measurements. They further advised us that it is necessary for us to adjust the dosing to accurately target the proposed

steady-state blood levels. Additional guidance from the FDA related to the regulatory pathway to be followed in gaining approval for marketing of the allopregnanolone drug product. We queried the FDA as to whether additional nonclinical studies would be required to support our IND filing and for registration of the drug product. We were informed that the FDA would not require additional nonclinical studies to support clinical trials provided the doses would not achieve plasma exposures exceeding 157 nM (or 50 ng/mL). At the time of NDA submission, reproductive and developmental toxicity studies would be required but these are not required to support the clinical trial.

IND Preparation, Filing and Approval. The initial IND document was submitted for review on September 6, 2011. The FDA acknowledged the date of receipt as September 13, 2011. The IND number assigned was 111,085. The FDA required additional detailed information on the procedure for drug product manufacturing; certificate of analyses for a representative batches of drug product, including assay, impurity/degradant levels, sterility results, pyrogenicity results, pH, osmolality and particulates; stability data and plans for continuing stability testing; storage details for the drug products; and a description of the infusion bags. This information was provided to the FDA on March 22, 2011. On May 7, 2012 the FDA approved the IND.

Case Report Forms and Databasing. A case report system was designed for the secure collection of data from the clinical trial using REDCap (Research Electronic Data Capture). A detailed template was prepared to capture data from each subject in the clinical trial. An extensive data set was collected on each subject. The data items collected include the following: 22 yes/no answers to questions relevant to the inclusion/exclusion criteria; demographic data on each subject (gender, race, ethnicity, language preference, education); informed consent information (type, date, time, confirmation); eligibility waivers; brain injury data; injuries and injury severity; Glasgow coma scale (pre-hospitalization, admission, randomization); pupillary reaction; abbreviated injury scale data; medical history information; secondary insults (hypoxic, hypotensive, inadvertent hypocapnia, cardiac arrest, seizures, clinical deterioration); cranial therapy (neurosurgical procedures); laboratory values (hematology, chemistry); CT scan data; neurological evaluations (daily and at follow-up visits); vital signs; body weight for dosing calculation; therapy intensity level (ICP therapy including CSF drainage, positioning, sedation, respirator settings, osmotic therapy, metabolic suppression with high dose barbiturates or propofol, neurosurgical procedures); ICP monitoring; ECG results; mortality information including causes; discharge details; study completion/termination; concomitant medications; adverse events; log of study dosing; times of blood draws for pharmacokinetic studies; EEG results; Glasgow outcome scale – extended (GOS-E) data; neuropsychological battery data; executive function tests data; learning tests data; working memory testing data; processing speed testing data; PHQ-9 (patient health questionnaire 9-item depression screen) data; SF-36 (36-item short form survey instrument) data; posttraumatic epilepsy data at 1 month and 3 month (responses to telephone questions concerning type, frequency, duration of seizures).

Data Safety Monitoring Board (DSMB). A chairperson and members of the DSMB were recruited and a DSMB Charter prepared. The DSMB Charter specified the DSMB objectives and responsibilities; lists the DMSB members; describes the responsibilities of the UC Davis Project Team, the responsibilities of the CTSC, and the responsibilities of the DSMB chairperson. It also describes DSMB meetings and voting including safety data review meetings and unscheduled

(ad-hoc) DSMB meetings. Procedures for identification of dose-limiting toxicities and identification of severe adverse events are described. The Charter also provides information on procedures for record retention and audit, the duration and changes to the DSMB membership, confidentiality, financial disclosure and conflict of interest disclosure, and provides a description of the information that should be stored in the Trial Master File.

Publication on ClinicalTrials.gov. On August 20, 2012, an entry on ClinicalTrials.gov, a registry of clinical trials operated by the National Library of Medicine was created for the trial. The ClinicalTrials.gov identifier is NCT01673828. Extensive information on the purpose, outcome measures, and eligibility criteria are included as well as key contacts. The University of California, Davis is listed as a sponsor and the Department of Defense is acknowledged.

Interactive Web Response System (IWRS). A web-based system was built for patient randomization, patient deactivation, GOS-E score recording, drug dispensation, drug shipment receipt, tracking of lost or damaged IV bags, and unblinding. A key aspect of the system is to provide web-based reports for study management and control of drug inventory. The system interfaces with the UC Davis Good Manufacturing Practices Laboratory and the Investigational Drug Services Pharmacy.

Institutional Review Board (IRB) Approval. The protocol was initially approved by the University of California, Davis IRB on May 10, 2012 and modifications were approved on June 13, 2012.

Human Research Protection Office (HRPO) Approval. The protocol was assigned HRPO Log Number A-15737 and reviewed by HRPO, a unit of the U.S. Army Medical Research and Materiel Command (USAMRMC). On July 18, 2012, notification was received that HRPO had determined that the protocol complies with applicable DoD, US Army and USAMRMC human subject protections requirements. HRPO initial approval was granted and was maintained for the duration of the trial.

Investigator's Brochure. An Investigator's Brochure was produced with information on the drug substance and drug product, including chemical properties, manufacturing and formulation. Drug related risks were summarized. Extensive pharmacology information was provided. A complete table of dosing based on body weight is also provided in this document.

Study Team, Study Monitoring and Quality Assurance. The study team included two medical monitors to share monitoring responsibilities, each providing coverage half-time. Since the study required active participation of the Emergency Department (ED), two ED physicians participated on the study team to supervise the ED staff and insure adequate recruiting and compliance with all study provisions at the point of entry into the study. Similarly, a neurosurgeon participated on the study team to coordinate the neurosurgical medical staff to insure active participation and compliance with all study requirements when study subjects are cared for by the neurosurgical service. Full time clinical research coordinator coverage was provided by two research coordinators who shared responsibilities of around the clock coverage. The clinical coordinators managed the implementation, quality control and completion of the trial, including patient recruitment. They provided a point-of-contact familiar with all aspects of the trial and provide

oversight and direction to ensure that all protocol and regulatory requirements were fully met in the conduct of the trial and that the data for each study subject was managed strictly according to the study protocol. The clinical research coordinators were assisted by a study assistant (junior specialist) who was available in the ED to ensure enrollment of all eligible and interested patients. The study assistant also assisted with informed consent and with blood collection and processing. The research coordinators and study assistant were supervised by a nurse supervisor with extensive experience in the conduct of TBI trials. This individual provided critical knowledge and judgment in the day-to-day operation of the trial and in patient recruitment.

A neuropsychologist supervised the conduct of the neuropsychological testing of study subjects. This individual was tasked with recruiting a psychometrician to conduct the neuropsychological testing. Additional personnel included in the study team were a senior supervising biostatistician responsible for the overall statistical conduct of the trial. A principal statistician was tasked with the responsibility for the conduct of the ongoing statistical analyses as the trial proceeded and for interaction with the DSMB.

An analytical chemist was responsible for chemical analytical studies in support of drug product manufacturing and bioanalytical support in the conduct of the clinical study. Among his responsibilities were analytical methods development and quality control of the drug product, and ongoing analysis of patient samples. The FDA had mandated ongoing plasma measurements to insure compliance with designated limits on exposure to the study drug. The analytical chemistry capability allowed this mandate to be fulfilled.

Initiation of Subject Enrollment. The study was opened for enrollment on May 17, 2013. Each enrolled subject was treated according to the study protocol. The study team monitored UC Davis Medical Center on an around-the-clock basis to ensure that any eligible patient with traumatic brain injury was provided an opportunity to participate in the study. The study team does not believe that any such patient escaped attention. However, many subjects failed to meet the inclusion criteria and were not able to be enrolled.

Subject Enrollment. The study enrolled 13 subjects. In these subjects, the Glasgow Coma Scale Scores (GCS) ranged from 3 to 11, with media score of 7; 11 subjects had GCS scores in the severe range (GCS 3–8) and 2 subjects had GCS in the moderate range (GCS 9–12). Demographic characteristics of the 13 enrolled subjects are shown in Table 8.

Table 8. Demographic Characteristics of Study Subjects

Characteristic	All Subjects (n = 13)	Placebo (n = 6)	Allopregnanolone (n = 7)
Age (Years)			
Mean (SD)	28.8 (13.0)	26.2 (13.5)	31.1 (13.1)
Median	24.0	21.5	29.0
Range	18.0–53.0	18.0–53.0	18.0–51.0
Age Category			
<40 N (%)	10 (76.9%)	5 (83.3%)	5 (71.4%)
>=40 N (%)	3 (23.1%)	1 (16.7%)	2 (28.6%)
Gender			
Female N (%)	2 (15.4%)	2 (33.3%)	0
Male N (%)	11 (84.6%)	4 (66.7%)	7 (100.0%)

TBI Severity			
Moderate N (%)	2 (15.4%)	0	2 (28.6%)
Severe N (%)	11 (84.6%)	6 (100.0%)	5 (71.4%)
Ethnicity			
Hispanic or Latino N (%)	4 (30.8%)	3 (50.0%)	1 (14.3%)
Not Hispanic or Latino N (%)	9 (69.2%)	3 (50.0%)	6 (85.7%)
Race			
Black N (%)	1 (7.7%)	1 (16.7%)	0
White N (%)	12 (92.3%)	5 (83.3%)	7 (100.0%)

The research team was notified of 185 potential subjects. Of these, 123 subjects had a moderate to severe TBI evident on the CT scan and were screened. Of those subjects screened, 109 were excluded and 13 were enrolled giving an enrollment rate was 10.7%. One eligible subject could not be enrolled because enrollment was closed due to HRPO review. The number of notifications, exclusions and enrollments by month are presented in Table 9.

Table 9. Number of Notifications, Exclusions and Enrollments by Month

Month	Notified	Excluded	Enrolled	% Enrolled
May 2013	4	3	1	25%
June 2013	14	13	1	7%
July 2013	12	10	2	17%
August 2013	15	14	1	7%
September 2013	14	14	0	0%
October 2013	9	8	1	11%
November 2013	6	4	2	33%
December 2013	8	6	2	25%
January 2014	8	8	0	0%
February 2014	10	10	0	0%
March 2014	7	6	1	14%
April 2014	11	11	0	0%
May 2014	12	12	0*	0%
June 2014	6	6	0	0%
July 2014	8	7	1	13%
August 2014	8	8	0	0%
September 2014	9	9	0	0%
October 2014	7	7	0	0%
November 2014	6	5	1	17%
December 2014	8	8	0	0%
January 2015	3	3	0	
Total	**185**	**172**	**13**	**7%**

*Closed for enrollment during HRPO review.

The reasons for exclusion among the 109 patients with moderate to severe TBI who were excluded are shown in the table below:

Table 10. Reasons for Subject Exclusion

Reason Not Enrolled	Number	Percent
Research staff not notified	2	2
Consent was declined	1	1
Unknown time of injury	26	24
No legally authorized representative available	29	27
Inclusion Criteria Not Met		
I1 – Post resuscitation GCS not 3–12	7	6
I2 – Age not 18–65 years	20	18
I3 – Not closed or blunt traumatic injury	5	5
I4 – Exceeded the enrollment time window (>8 hours)	15	14
I5 – Not able to participate for the full term of study (12 months)	7	6
Met Exclusion Criteria		
E1 – Subject does not speak English or Spanish	2	2
E2 – Life expectancy less than 24 hours per Investigator	4	4
E3 – Bilateral non-reactive pupils with GCS 3	9	8
E4 – Isolated epidural hematoma	0	0
E5 – Hypoxia: pulse oximetry < 90% for 15 or more minutes before enrollment	4	4
E6 – Hypotension: systolic BP <90 mmHg on two or more reliable measurements before enrollment	11	10
E7 – Cardiopulmonary arrest prior to randomization	10	9
E8 – Spinal cord injury with motor defects	0	0
E9 – Positive pregnancy test	0	0
E10 – Pre-existing significant disease that could affect outcome	4	4
E11 – Prisoner/ward of the state	7	6
E12 – Active cancer of the breasts or reproductive organs	0	0
E13 – History of thromboembolic events	0	0
E14 – Received activated Factor VII before enrollment	3	3
E15 – Allergy to progesterone	0	0
E16 – Any disease that, in the opinion of the Investigator, is unstable or which could jeopardize the safety of the patient	4	4
E17 – Treatment with another investigational drug or procedure within the last 30 days	1	1
E18 – Body weight > 120 kg	2	2
E19 – Severe renal impairment (creatinine clearance < 50 mg/mL)	2	2

The research team was notified on a timely basis of all patients evaluated at UC Davis Medical Center who had sustained a moderate or severe TBI. For the 13 enrolled subjects, the mean time to research team notification was 58 min (range, 7 to 99 min); the mean time to randomization was 261 min (range, 174 to 328 min); and the mean time to study drug administration was 370 min (range, 287 to 441 min). In all cases where a subject was enrolled in the trial, treatment with study drug and blood and data collection proceeded as per protocol.

Adverse Events. Table 11 provides a summary of treatment-emergent adverse events (TEAEs) separated by placebo and active drug (allopregnanolone). As noted in the table, all subjects experienced TEAEs that were considered severe but they were considered not likely to be related to study drug treatment. One subject receiving treatment died; the death occurred in a subject receiving active drug but was not considered related to the study treatment.

Table 11. Treatment-Emergent Adverse Events (TEAEs) by Severity, Relatedness, Death and Discontinuation in Placebo and Allopregnanolone Groups

Adverse Event Category	Placebo (n=6) N (%)	Allopregnanolone (n=7) N (%)
N (%) of Subjects with TEAEs		
All AE's	6 (100%)	7 (100%)
Treatment-Related AEs	0	0
N (%) of Subjects With TEAEs By Maximum Severity		
All AE's		
Severe	6 (100%)	7 (100%)
Treatment-Related AEs		
Severe	0	0
N (%) of Subjects with TEAEs Resulting in Death		
All AE's	0	1 (14.3%)
TEAEs	0	0
N (%) of Subjects with TEAEs Resulting in Discontinuation		
All AEs	0	0
Treatment-Related TEAEs	0	0

Table 12 provides a summary of treatment-emergent adverse events (TEAEs) by Medical Dictionary of Regulatory Activities (MedDRA) system organ class, preferred term in the treated population (both placebo and active drug). If a subject has more than one TEAE that codes to the same preferred term, the subject was counted only once for that preferred term. Similarly, if a subject had more than one treatment-emergent adverse event within a system organ class category, the subject was counted only once in that system organ class category using the event with highest severity. As noted in the table, none of the TEAEs were considered related to the study treatment.

Table 12. Treatment-Emergent Adverse Events (TEAEs) by MeDRA Term

System Organ Class Preferred Term	Unrelated N (%)	Related N (%)	All AEs N (%)
At Least One AE	13 (100%)	0	13 (100%)
Blood and lymphatic system disorders	3 (23%)	0	3 (23%)
Anaemia	2 (15%)	0	2 (15%)
Jaundice	1 (8%)	0	1 (8%)
Cardiac disorders	4 (31%)	0	4 (31%)
Bradycardia	1 (8%)	0	1 (8%)
Ischaemic cardiomyopathy	1 (8%)	0	1 (8%)
Pulmonary oedema	2 (15%)	0	2 (15%)
Tachycardia	1 (8%)	0	1 (8%)
Eye disorders	1 (8%)	0	1 (8%)
Conjunctival oedema	1 (8%)	0	1 (8%)
General disorders and administration site conditions	2 (15%)	0	2 (15%)
Hypothermia	1 (8%)	0	1 (8%)
Pyrexia	1 (8%)	0	1 (8%)
Alcohol withdrawal symptom	1 (8%)	0	1 (8%)
Infections and infestations	6 (46%)	0	6 (46%)
Fungal infection	2 (15%)	0	2 (15%)
Respiratory tract infection	2 (15%)	0	2 (15%)
Staphylococcal sepsis	1 (8%)	0	1 (8%)

Urinary tract infection	1 (8%)	0	1 (8%)
Urinary tract infection	1 (8%)	0	1 (8%)
Injury, poisoning and procedural complications	9 (69%)	0	9 (69%)
Cerebral hypoperfusion	9 (69%)	0	9 (69%)
Investigations	7 (54%)	0	7 (54%)
Central venous pressure decreased	1 (8%)	0	1 (8%)
Electrocardiogram QTc interval prolonged	6 (46%)	0	6 (46%)
Electrocardiogram ST segment elevation	1 (8%)	0	1 (8%)
Electrocardiogram T wave inversion	1 (8%)	0	1 (8%)
Internation normalised ratio increase	1 (8%)	0	1 (8%)
Metabolism and nutrition disorders	6 (46%)	0	6 (46%)
Diabetes insipidus	3 (23%)	0	3 (23%)
Hypervolemia	2 (15%)	0	2 (15%)
Hyponatremia	2 (15%)	0	2 (15%)
Musculoskeletal and connective tissue disorders	2 (15%)	0	2 (15%)
Compartment syndrome	2 (15%)	0	2 (15%)
Nervous system disorders	12 (92%)	0	12 (92%)
Craniocerebral injury	1 (8%)	0	1 (8%)
Headache	1 (8%)	0	1 (8%)
Intracranial pressure increase	12 (92%)	0	12 (92%)
Meningitis	1 (8%)	0	1 (8%)
Neuralgia	1 (8%)	0	1 (8%)
Spinal epidural hematoma	2 (15%)	0	2 (15%)
Psychiatric disorders	6 (46%)	0	6 (46%)
Agitation	5 (38%)	0	5 (38%)
Drug withdrawal syndrome	1 (8%)	0	1 (8%)
Renal and urinary disorders	1 (8%)	0	1 (8%)
Polyuria	1 (8%)	0	1 (8%)
Respiratory, thoracic and mediastinal disorders	12 (92%)	0	12 (92%)
Acute respiratory distress syndrome	2 (15%)	0	2 (15%)
Bronchospasm	3 (23%)	0	3 (23%)
Hypoxia	2 (15%)	0	2 (15%)
Pneumonia	4 (31%)	0	4 (31%)
Pneumonia aspiration	3 (23%)	0	3 (23%)
Pneumonia staphylococcal	3 (23%)	0	3 (23%)
Pneumothorax	2 (15%)	0	2 (15%)
Sinusitis	1 (8%)	0	1 (8%)
Skin and subcutaneous tissue disorders	1 (8%)	0	1 (8%)
Rash	1 (8%)	0	1 (8%)
Vascular disorders	10 (77%)	0	10 (77%)
Deep vein thrombosis	1 (8%)	0	1 (8%)
Haemorrhage	3 (23%)	0	3 (23%)
Hypertension	4 (31%)	0	4 (31%)
Hypotension	8 (62%)	0	8 (62%)
Verbatim terms not coded	7 (54%)	0	7 (54%)
Blood infection	1 (8%)	0	1 (8%)
Corneal ulcers	1 (8%)	0	1 (8%)
Forearm eschar	1 (8%)	0	1 (8%)
Paroxysmal sympathetic hyperactivity	1 (8%)	0	1 (8%)
Pulmonary embolism/ deep vein thrombosis	1 (8%)	0	1 (8%)
Sacrum ulcer	1 (8%)	0	1 (8%)
Submandibular abcess	1 (8%)	0	1 (8%)

Tracheotomy site infection	1 (8%)	0	1 (8%)
Tracheotomy site necrosis	1 (8%)	0	1 (8%)

The enrolled subjects experienced a total of 15 serious adverse events (SAEs). None of these were considered related to the study drug. The specific SAEs are given in Table 13.

Table 13. Serious Adverse Events (SAEs)

SAE Term	Number
Leg compartment syndrome	2
Acute respiratory distress syndrome	2
Epidural hematoma	2
Hemorrhage	1
Hypotension	1
Aspiration pneumonia	1
Increased intracranial pressure	2
Worsening traumatic brain injury	1
Hypoxia	1
Traumatic brain injury	1
Brain herniation (transtentorial and uncal)	1

The incidences of adverse events by MedDRA system organ class preferred term occurring at least 20% more frequently in the placebo subjects are shown in Table 14.

Table 14. Adverse Events Occurring at least 20% More Frequently in Placebo Arm

System Organ Class Preferred Term	Placebo (n=6) N (%)	Allopregnanolone (n=7) N (%)
Anemia	2 (33%)	0 (0%)
Pulmonary edema	2 (33%)	0 (0%)
Fungal infection	2 (33%)	0 (0%)
Electrocardiogram QTc interval prolonged	4 (67%)	2 (29%)
Hypervolemia	2 (33%)	0 (0%)
Acute respiratory distress syndrome	2 (33%)	0 (0%)
Pneumonia aspiration	3 (50%)	0 (0%)
Hypertension	3 (50%)	1 (14%)

The incidences of adverse events by MedDRA system organ class preferred term occurring at least 20% more frequently in the subjects receiving active drug are shown in Table 15.

Table 15. Adverse Events Occurring at least 20% More Frequently in Allopregnanolone Arm

System Organ Class Preferred Term	Placebo (n= 6) N (%)	Allopregnanolone (n=7) N (%)
Infection	0 (0%)	2 (29%)
Hyponatremia	0 (0%)	2 (29%)
Compartment syndrome	0 (0%)	2 (29%)
Pneumonia	0 (0%)	4 (57%)
Pneumothorax	0 (0%)	2 (29%)
Hypotension	3 (50%)	5 (71%)

The study investigators did not attribute any of the adverse events to either placebo or allopregnanolone.

The incidences of protocol-defined clinical and laboratory adverse events occurring at least 20% more frequently in the placebo arm are shown in Table 16.

Table 16. Protocol-defined Clinical and Laboratory Adverse Events Occurring at Least 20% More Frequently in Placebo Arm

Protocol Defined Adverse Event	Placebo (n=6) N (%)	Allopregnanolone (n=7) N (%)
Clinical		
Infection (localized to systemic)	5 (83.3%)	3 (42.9%)
Prolonged QTcF interval (≥0.45 sec)	4 (66.7%)	2 (28.6%)
Laboratory		
International Normalized Ratio (INR), prolonged (≥1.1 ULN)	2 (33.3%)	0
Platelets, decreased (≤125,000/mm^3)	3 (50.0%)	2 (28.6%)

A placebo subject exhibited the following potentially life threatening laboratory adverse event: cardiac troponin I levels consistent with myocardial infarction or unstable angina.

The incidences of protocol-defined clinical and laboratory adverse events occurring at least 20% more frequently in the allopregnanolone arm are shown in Table 17.

Table 17. Protocol-defined Clinical and Laboratory Adverse Events Occurring at Least 20% More Frequently in Allopregnanolone Arm

Protocol Defined Adverse Event	Placebo (n= 6) N (%)	Allopregnanolone (n= 7) N (%)
Clinical		
Alteration in personality – behavior or mood (agitation)	1 (16.7%)	3 (42.9%)
Laboratory		
AST, elevated (≥ 1.25 ULN)	0	5 (71.4%)
Absolute lymphocyte count, low (<650/mm^3)	2 (33.3%)	4 (57.1%)
Bilirubin (total), elevated (≥1.1 ULN)	0	2 (28.6%)
Magnesium, serum, low (< 1.7 mg/dl)	3 (50.0%)	6 (85.7%)
Partial Thromboplastin Time (PTT), prolonged (≥1.1 ULN)	0	2 (28.6%)

Allopregnanolone subjects exhibited the following potentially life threatening laboratory adverse events: transfusion of more than 2 units of packed RBCs, and shock requiring vasopressors to maintain blood pressure.

Pharmacokinetic Results. We used pharmacokinetic modeling to determine the dosing required to achieve the target blood levels as defined in the protocol submitted to the FDA and on which the conduct of the study is based. Our modeling used a limited set of data available in the literature. However, allopregnanolone has not previously been administered to human subjects in the treatment of a disease. More specifically, allopregnanolone has not been administered to human subjects who have experienced TBI. Therefore, it was necessary to rigorously monitor the plasma levels achieved with our dosing regimen to verify that the dosing regimen we selected is

adequate. Blood draws for plasma allopregnanolone measurements were completed on all enrolled subjects. Figure 9 shows the plasma allopregnanolone levels in the 6 subjects receiving active drug in the cohort of 12 subjects in the component of the study receiving Product L (0.5 mg/mL allopregnanolone in 6% cyclodextrin in 0.9% sodium chloride injection, USP) intended to target a 50 nM steady-state plasma allopregnanolone concentration.

Figure 9. Plasma Allopregnanolone Levels in 6 Subjects Receiving Infusion with Product L

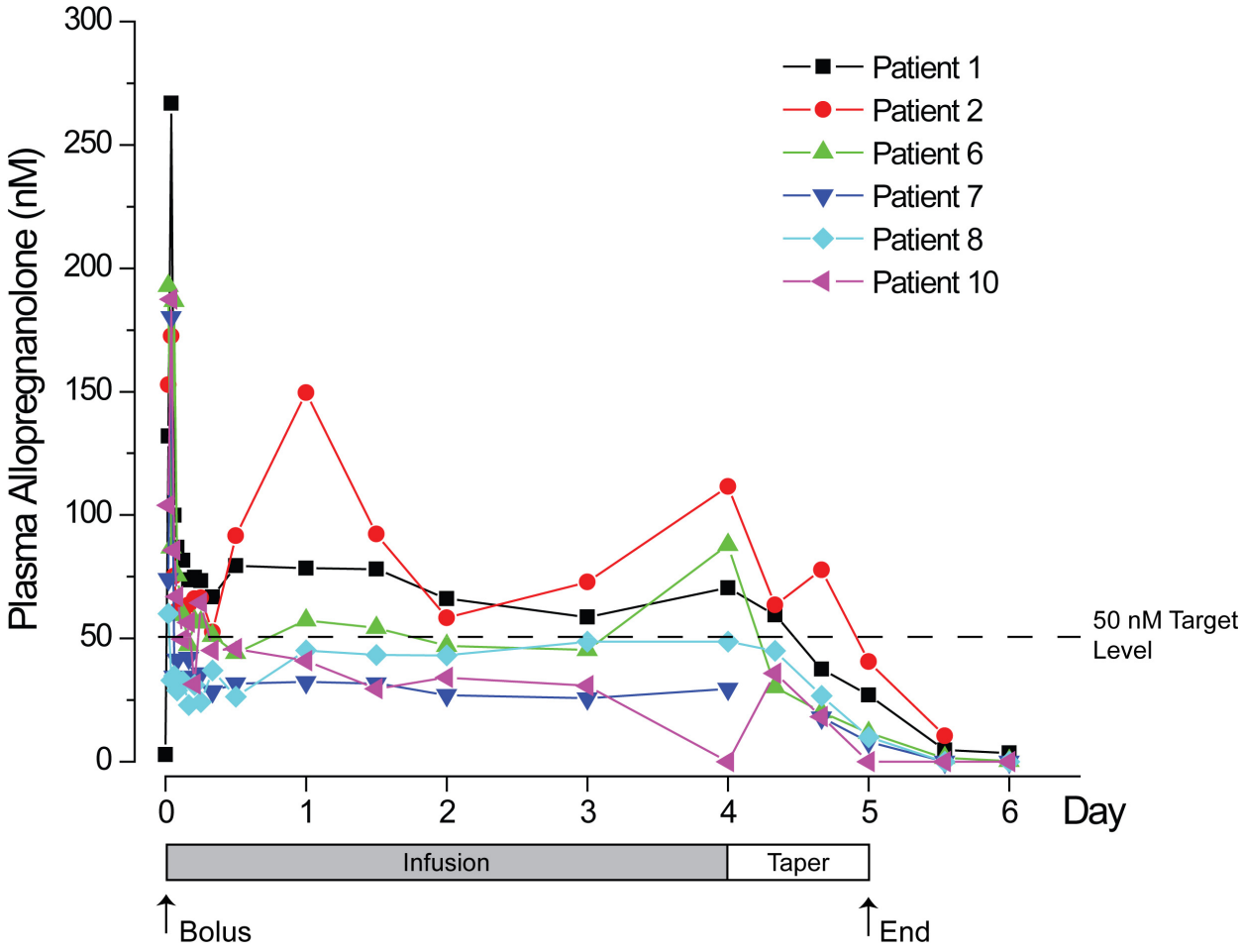
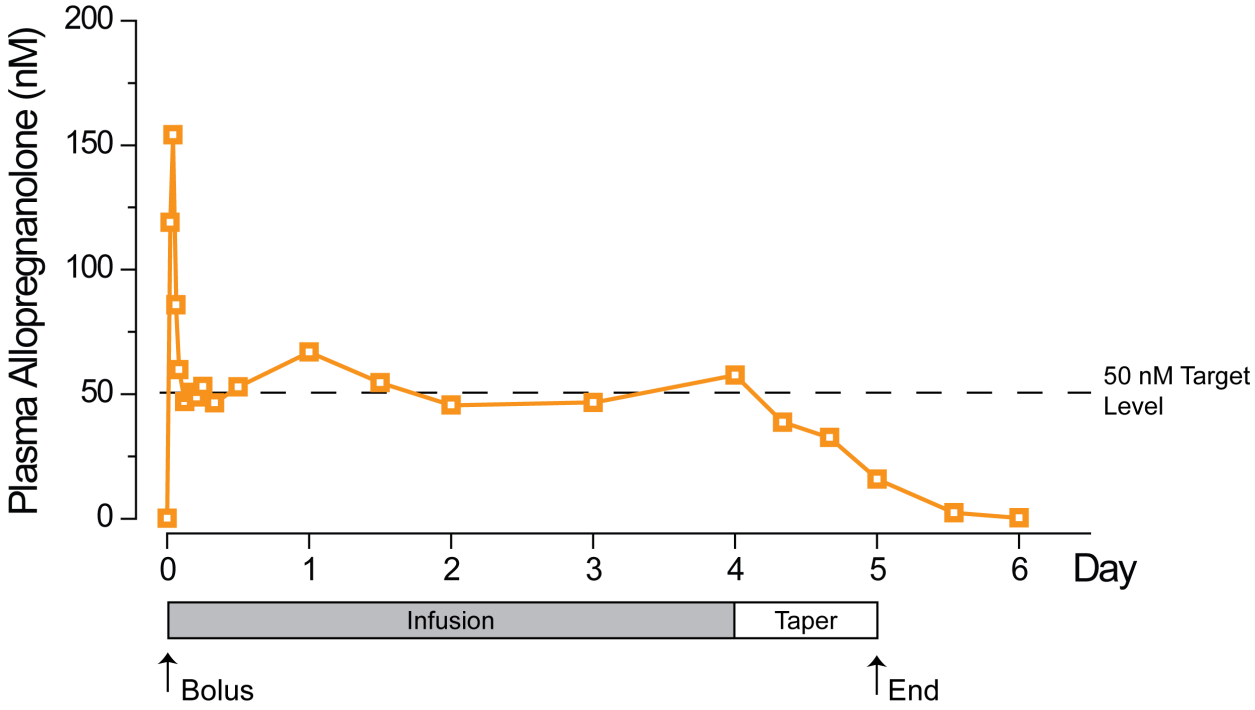


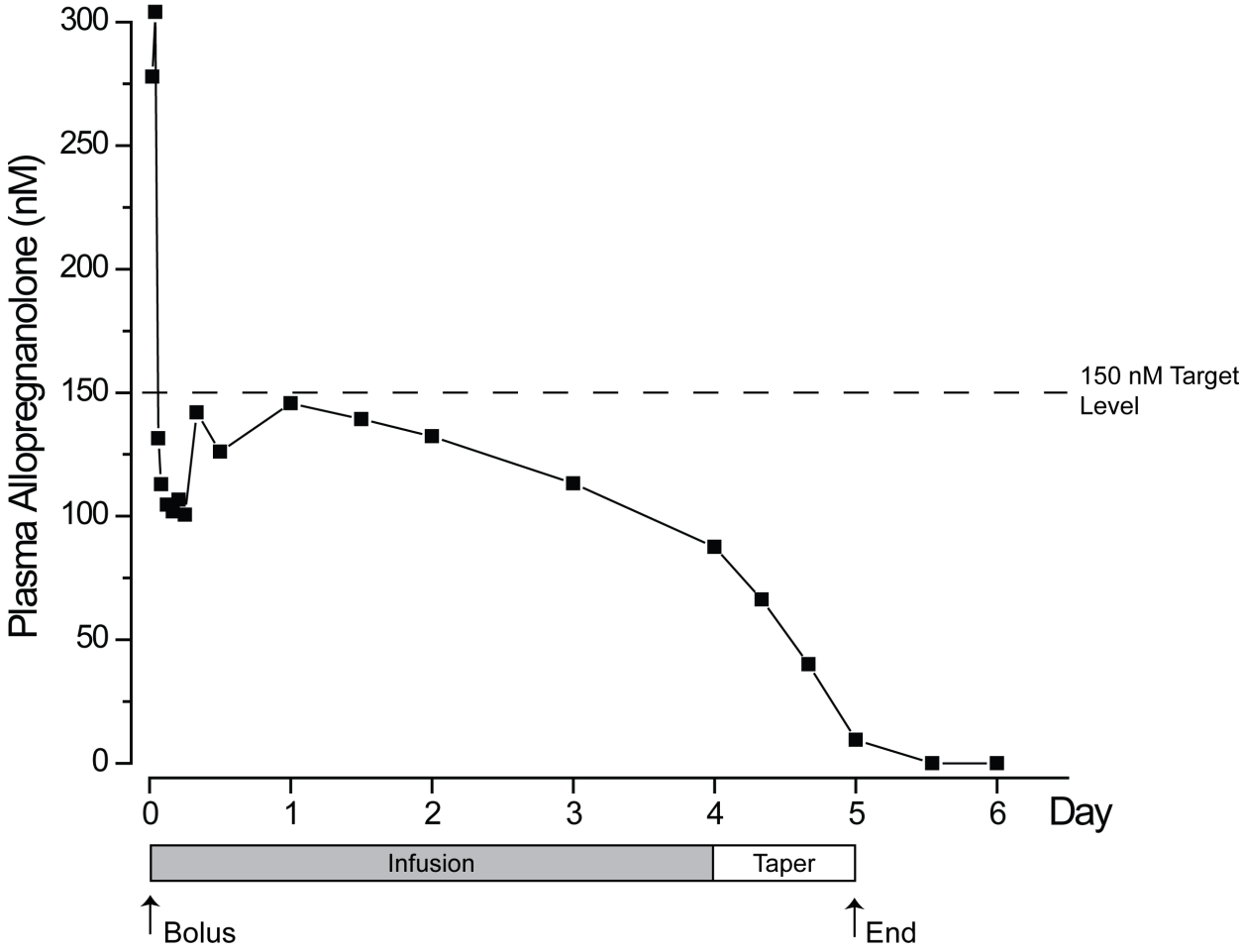
Figure 10 shows the mean plasma allopregnanolone level values at each time point for the 6 subjects receiving the Product L infusion. It is apparent that allopregnanolone plasma concentrations initially overshoot the 50 nM target level during the 1 hour bolus infusion but then return to the target level where they remain until the taper begins. In these 6 subjects, we determined the steady-state plasma allopregnanolone concentrations in 6 blood draws obtained from 12 to 96 hours after dosing, when plasma levels were relatively steady. The geometric mean plasma concentration in these 6 subjects was 49.5 nM, which closely matches the target level we intended to achieve of 50 nM. We therefore conclude that the dosing regimen selected is adequate.

Figure 10. Mean Plasma Allopregnanolone Level Values for 6 Subjects Receiving Infusion with Product L as Shown in Figure 9



Following review of the steady-state plasma level data in the first cohort of 12 subject in conjunction with assessment of the available safety information demonstrating a lack of adverse events attributed to the active treatment, the DSMB gave approval to enroll subjects to receive Product H, high dose formulation consisting of 1.5-mg/mL allopregnanolone in 6% cyclodextrin in 0.9% sodium chloride injection, USP, which was intended to target a 150 nM plasma concentration. One subject received dosing with Product H. Plasma allopregnanolone levels from this subject are shown in Figure 11. As was the case for subjects treated with Product L, there is an initial overshoot during the 1-hour bolus infusion. Plasma levels then fall, undershooting the target but rebound to nearly match the target concentration on Day 1. The concentration drifts downward despite continued infusion until the taper begins on the last day of the infusion. The basis for the downward drift was not defined.

Figure 11. Plasma Allopregnanolone Levels in Subject Receiving Infusion with Product H



Population Pharmacokinetics. A population pharmacokinetic analysis was conducted using data from 5 evaluable subjects who received low dose allopregnanolone infusions (Product L) to target 50 nM steady-state plasma levels. Data from the sixth subject could not be included in the analysis as the subject was clinically unstable and expired. A two-compartment pharmacokinetic model was used for the analysis with an effect-compartment link as shown in Figure 12. Physiologic pharmacokinetic parameters derived from the model are shown in Table 18. The following relationships are used $CL = V_1 \bullet K_{10}$ or $K_{10} = CL/V_1$, where CL is the clearance. An inter-compartmental clearance parameter Q was defined by the following: $K_{12} = Q/V_1$ and $K_{21} = Q/V_2$. The following relationships also hold: V_{ss} [L/kg] = V_1 [L/kg] + V_2 [L/kg]; amount in body at steady state infusion [μg/kg] = C_{ss} [ng/mL] • V_{ss} [L/kg] and steady state concentration [ng/mL] = infusion rate [μg/kg/hr]/CL [L/kg/hr].

Figure 12. Two-compartment Linear Population Pharmacokinetic Model

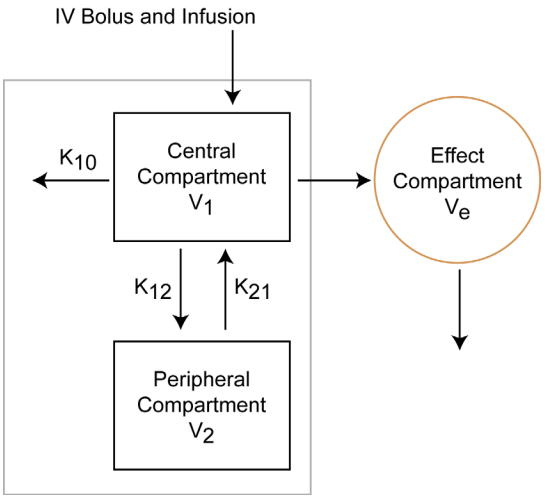


Table 18. Mean Population Parameter Estimates Based on Pharmacokinetic Data from 5 Evaluable Subjects Receiving Product L

Final Model Population Parameter Estimates					
Model: pk001no2np.nmi					
				95% CI Limits *	
Parameter	Estimate	SEE	%CV	Lower Limit	Upper Limit
CL, L/kg/hr.	1.79	0.0811	4.5	0.929	1.94
Q, L/kg/hr	0.145	0.077	53.1	0.0529	0.946
V1, L/kg	0.869	0.136	15.7	0.534	1.4
V2, L/kg	2.6	0.428	16.5	2.1	4.91
Residual Error % RSD	40.1	3.43	8.6	3.31	4.62
Alpha Half-Life, hr. **	0.31			0.178	0.487
Beta Half-Life, hr. **	13.2			8.3	55.6
Rate for Css=50 nM, mcg/kg/hr	28.5			14.8	30.9
Rate for Css=150 nM, mcg/kg/hr	85.5			44.4	92.7
* Based on N=1000 parametric bootstrap. For V2 and Beta Half-Life the 80% confidence intervals are presented					
** Median and confidence intervals calculated using results of N=1000 parametric bootstrap					

Subject Follow-Up. Good follow-up of the enrolled subjects was obtained. Of the 13 enrolled subjects, 12 entered follow-up and 1 died after withdrawal of care on study day 6 but prior to the 1 month evaluation. Table 19 provides a summary of the follow-up status for all enrolled subjects.

Table 19. Subject Follow-up Encounters

Subject	Day 6	1 Month	3 Month	6 Month	12 Month
001-001	X	X	X	X	X
001-002	X	Deceased	Deceased	Deceased	Deceased
001-003	X	X	X	X	X
001-004	X	X	X	X	X
001-005	X	X	X	X	X

001-006	X	X	X	X	X
001-007	X	X	X	O	X
001-008	X	X	X	O	X
001-009	X	X	X	X	X
001-010	X	X	X	X	X
001-011	X	X	X	X	X
001-012	*	Deceased	Deceased	Deceased	Deceased
001-013	X	X	X	X	X

X=Completed; O=Opted out; *Care withdrawn during Study Day 6

Efficacy Outcome. Table 20 summarizes the efficacy outcomes in the 12 subjects randomized to Product L. The primary outcome measure of the clinical study was GOS-E at 6 months after injury. There was a wide range of outcomes among the subjects in both groups nearly spanning the 8-point GOS-E scale from death (1) to lower good recovery (7). There was no statistically significant difference in the mean outcome values in the two groups but the number of enrolled subjects is too small to draw any conclusion about the efficacy of the treatment. Only one subject received Product H.

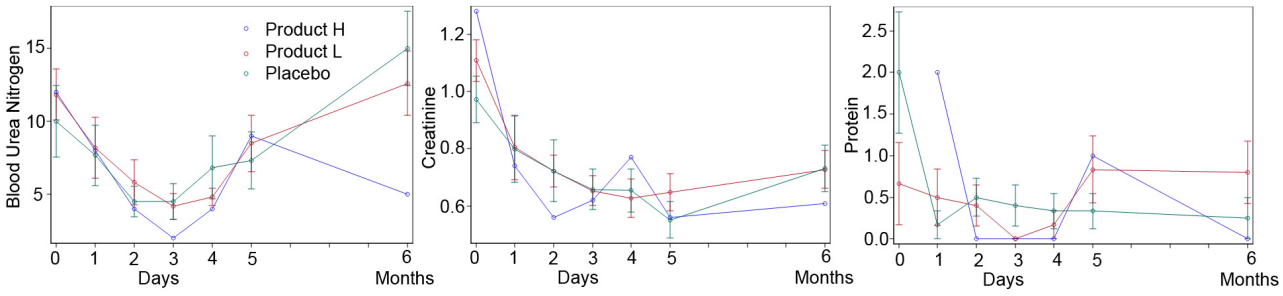
Table 20. Mean GOS-E Values at 6 Months After Injury in Subjects Receiving Placebo or Product L

	Placebo (N=6)	Allopregnanolone (N=6)
Mean	4.3	4.2
SD	1.8	1.9
Range	1 (death) to 6 (upper moderate disability)	1 (death) to 7 (lower good recovery)

*GOS-E is an 8-point scale, ranging from death (1) to upper good recovery (8).

Laboratory Testing. According to protocol, extensive laboratory testing conducted during the course of the study. Electrocardiograms and electroencephalograms were obtained. Blood was drawn at time of screening, during each day of treatment, and at the 6-month follow-up visit. No changes in any of the laboratory values were attributed to the study drug. The graphs in Figure 13 plot the mean blood urea nitrogen and creatinine and urine protein values in the active drug and placebo groups. These values are of particular interest because of the potential of the cyclodextrin excipient in all product formulations (including placebo) to adversely affect renal function. As seen in the graphs, there is no evidence that the products acutely impact renal function.

Figure 13. Mean Blood Urea Nitrogen and Creatinine and Urine Protein Values in Subject Receiving Product H, Product L or Placebo



Values are in mg/dL.

Section III – Key Research Accomplishments

Product Development

- Developed synthetic method for large scale manufacturing of pharmaceutical grade (GMP) allopregnanolone (API)
- Manufactured API according to GMP standards, providing the only domestic source of this material that meets FDA requirements
- Assured purity of API to meet FDA standards
- Assured stability of API to meet FDA standards
- Developed chemical analytical assay (HPLC) and bioanalytical methodology (UPLC-MS/MS)
- Developed intravenous formulations that met USP standards for osmolality, pH and particulates and was approved by FDA for investigational use in the present study
- Created three dosage forms (placebo and two active drug products to target low and high steady-state blood levels)
- Assurance of stability of formulation
- Prepared CMC document for allopregnanolone API and drug product meeting FDA standards

Clinical Trial Development

- Conducted pharmacokinetic modeling to define dosing
- Developed trial design and clinical protocol
- Developed statistical analysis scheme, adaptive protocol design, case report system, database
- Developed intravenous dosing scheme (bolus and continuous infusion) that provides for rapid achievement of target blood level and maintenance of levels during the treatment period

Regulatory

- Prepared IND and received FDA approval to proceed with trial
- Obtained IRB and HRPO approval to proceed with trial and successfully passed periodic continuing review
- Published record of study on ClinicalTrials.gov

Clinical Trial Conduct

- Recruited subjects into trial
- Successfully dosed active treatments and placebo
- Collected safety and efficacy data
- Collected blood and performed pharmacokinetic analyses

Clinical Trial Outcome

- Verified dosing based on pharmacokinetic modeling
- Obtained evidence of tolerability and safety of active drug treatment
- No adverse events attributed to active drug treatments

Section IV – Reportable Outcome

Publications describing the results of the research are in preparation.

Section V – Conclusion

The research conducted in this project has advanced the development of allopregnanolone as a potential treatment for TBI. Prior to the initiation of this project, allopregnanolone had not been administered to humans for the treatment of any disease and it was not approved by the FDA for investigational use in humans. Following an intensive effort, a synthetic route for manufacturing pharmaceutical grade allopregnanolone was identified. The manufacturing methods discovered and implemented in this research can be applied by others who seek to manufacture allopregnanolone for clinical trials in TBI. The methods are also applicable to the eventual production of allopregnanolone for deployment as a treatment agent if approved for clinical use by regulatory authorities. In addition to creating a method for preparing the GMP grade API, a novel intravenous product formulation was developed that is practical, safe and applicable for clinical use in the treatment of TBI. Allopregnanolone is a highly hydrophobic substance that is not soluble by itself in an aqueous mediium. An intravenous product form that is clinically applicable requires the API to be solubilized. This was achieved in the present project using a cyclodextrin. The development of a clinically applicable formulation in which allopregnanolone is stably dissolved represents a substantial achievement. A key requirement of the product is that it is sufficiently stable to be deployed in military field use. Extensive testing was conducted to select a container compatible with the product formulations that permits long-term storage. Assays of the products were carried out by high performance liquid chromatography (HPLC) and we determined the pH, osmolality, and particulates according to USP requirements, as mandated by the FDA. Certificates of analysis were generated. Stability testing was also conducted as required by the FDA. An additional achievement is the successful development of a dosing regimen that achieved the objective of raising plasma allopregnanolone levels to the defined therapeutic targets. Furthermore, a validated bioanalytical method was developed for the measurement of allopregnanolone in human blood plasma that utilizes an ultrahigh pressure liquid chromatograph (UPLC) system and tandem quadrapole mass spectrometer (MS/MS).

An additional component of this project was the development of a rigorous clinical trial design, including an intravenous dosing scheme based on pharmacokinetic modeling. All regulatory requirements were met to initiate administration of the investigational agents in the context of a masked, placebo-controlled clinical trial. Overall, a total of 13 subjects that had sustained moderate to severe TBI were enrolled in the trial. Pharmacokinetic analyses demonstrated that the dosing scheme was adequate. None of the subjects experienced adverse events attributed to the study drug formulations.

In sum, the research conducted under this award has advanced the development of allopregnanolone as a potential drug treatment for TBI. Allopregnanolone product forms were created that are FDA approved for investigational use under an IND. The approved IND defines the regulatory requirements for the allopregnanolone product forms and for the clinical study of allopregnanolone. Pharmacokinetic studies conducted in the context of the trial provide a basis for dosing of allopregnanolone in future trials. No safety concerns were raised by the clinical

trial. Further research is required to determine if allopregnanolone is efficacious as a treatment for TBI.

Section VI – References

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Appendix I – Active Pharmaceutical Ingredient Specification

SAFC PHARMA **SPECIFICATION**
Madison, WI 53711 **Dept. 200I**
Page: 1

Allopregnanolone (UCD)

PRODUCT ID: UCD **FORMULA:** $C_{21}H_{34}O_2$ **MW:** 318.49

CAS NUMBER: NA **CATEGORY:** 4

SAMPLING: **QC TEST:** 2.0g
2.0g for microbial limits
200mg for bacterial endotoxin
– Use sterile containers for microbial limits and bacterial endotoxin sampling
RESERVE: 8.4g

STORAGE: Store at controlled room temperature

RETEST: 1 year from date of manufacture

PACKAGING

Product: Primary: Polyethylene charge bag with gasket, end cap, and clamp, size appropriate
Secondary: 4 mil Polyethylene bag, size appropriate
Tertiary: 30 Gallon open top plastic drum, RMS-006129

QC Sample: Primary: Type III amber glass jar with Teflon lined screw cap, size appropriate
Secondary: 4 mil Polyethylene bag, size appropriate

Reserves: Same as product packaging configuration

SPECIFICATIONS

APPEARANCE:	White to off-white powder
IDENTIFICATION (FT-IR):	Conforms to standard
^{1}H NMR:	Conforms to standard
IDENTIFICATION (HPLC):	Retention time ratio within 0.98 to 1.02 of standard
ASSAY (HPLC):	NLT 98.0%
IMPURITIES (HPLC):	No impurities detected greater than or equal to 1.0%
RESIDUAL SOLVENTS:	Methanol: NMT 3000ppm Dichloromethane: NMT 600ppm 2-Methyl-tetrahydrofuran: NMT 5000ppm Isopropyl alcohol: NMT 5000ppm Tetrahydrofuran: NMT 720ppm Heptane: NMT 5000ppm Ethyl acetate: NMT 5000ppm
WATER CONTENT:	Report
RESIDUE ON IGNITION:	NMT 0.1%

DSC:	Report onset
HEAVY METALS (ICP-MS):	Palladium: NMT 50 ppm NMT 10 ppm total USP metals (mercury, lead, bismuth, arsenic, antimony, tin, cadmium, silver, copper and molybdenum)
MICROBIAL LIMITS:	Total aerobic microbial count: NMT 100 CFU/g
BACTERIAL ENDOTOXIN:	NMT 1.0 EU/mg

TESTING

APPEARANCE: 200-100

IDENTIFICATION (FT-IR): 200-227
Use KBr HATR method.

^{1}H NMR: 200-221
Use $CDCl_3$ as the solvent.

IDENTIFICATION, ASSAY, and IMPURITIES (HPLC): OP-018724

RESIDUAL SOLVENTS: 200-992

WATER CONTENT: 200-232
Analyze a sample of approximately 100-150mg dissolved in 4.0ml methanol using the solution technique. Perform in triplicate with AG reagent.

RESIDUE ON IGNITION: 200-248; <USP 281>
Use approximately 1.0g sample.

DSC: 100-232
Method conditions: Ramp from 25-350°C at 10°C/min with N_2 at 50mL/min.

HEAVY METALS (ICP-MS):
Send approximately 250mg sample for analysis of palladium and USP metals (mercury, lead, bismuth, arsenic, antimony, tin, cadmium, silver, copper and molybdenum) to Exova at 9240 Santa Fe Springs Road, Santa Fe Springs, CA, 90670. Reference Exova Job No. 125857 on the test request form.

MICROBIAL LIMITS: USP <61>
Send approximately 2.0g to SGS Life Science Services at 616 Heathrow Drive, Lincolnshire, IL, 60069. Request "Total aerobic microbial count" to be performed per USP <61>. Include the Assignment No. 0981010325 on the test request form.

BACTERIAL ENDOTOXIN: USP <85>
Send approximately 200mg to Associates of Cape Cod at 124 Bernard E. St. Jean Drive, East Falmouth, MA, 02536. Request testing to be performed per IC Number 1010-063.

SHIPPING INSTRUCTIONS Per OP 500-401
For IATA, classify as:
Toxic solid, organic, n.o.s.
Allopregnanolone
UN 2811, Packaging Group II

Appendix IIA – Certificate of Analysis of Analysis of Qualification Run Material

SAFC™ Inspiring Science

645 Science Dr., Madison, WI 53711
Tel (608) 233-3115 Fax (608) 233-6873

CERTIFICATE OF ANALYSIS v.4

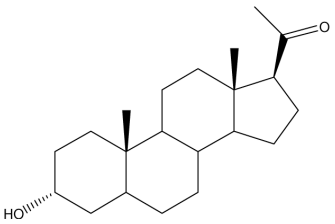
Material: Allopregnanolone (UCD)

Lot No.: 1010398010

Empirical Formula: $C_{21}H_{34}O_2$

Molecular Weight: 318.49

Structure:



Appearance:	White powder
HPLC Purity:	98.56% Impurities: RRT 0.06 = 0.22% RRT 0.78 = 0.55% RRT 0.94 = 0.52% RRT 1.10 = 0.17%
DSC Melt:	Onset: 174.9°C
^{1}H NMR:	Consistent with structure
FT-IR:	Consistent with structure
Water:	< 0.15%
Residual Solvents:	Methanol: Not detected Isopropyl Alcohol: Not detected Dichloromethane: Not detected Tetrahydrofuran: 13ppm Ethyl Acetate: 47ppm 2-Methyl THF: Not detected Heptane: 131ppm
Pd, ICP-MS:	Not detected (< 0.1ppm)
Heavy Metals, ICP-MS:	Cu: 0.26ppm Mo: 0.08ppm Ag: 0.06ppm All other elements (Sb, As, Bi, Cd, Pb, Hg, Sn) Not detected (< 0.1ppm each)
Residue on Ignition:	< 0.1%
Endotoxin:	< 5 EU/mg
Microbial Limits:	< 50 CFU/g

Purity Factor = 98.54% (100 - water – total solvents – ROI)* HPLC area % [(100-0-0.0191-0)*0.9856

Storage: Store at ambient room temperature.

Handling: Exercise caution in the handling and formulation of this product.

Manufacture Date: October 13, 2010 **Retest Date:** October 2011

Note: This material is for Research and Development use only and is not intended for use in humans. SAFC assumes no liability for damage resulting from handling, contact or misuse of this product.

11/16/10
TMJ/CMH

Appendix IIB – Certificate of Analysis of Analysis of GMP Grade Material

SAFC Pharma™
Inspiring Science

645 Science Drive, Madison, WI 53711 USA
Tel: (608) 233-3115 Fax: (608) 233-6873
www.safcglobal.com

CERTIFICATE OF ANALYSIS

Material:	**Allopregnanolone (UCD)**	**SAFC Lot:**	**1011UCD01**
Empirical Formula:	**$C_{21}H_{34}O_2$**	**Formula Weight:**	**318.49**
Manufacture Date:	**November 11, 2010**	**Retest Date:**	**November 11, 2011**

	Specification FPS-010300 v.2	**Results**
APPEARANCE: OP 200-100 v.8	White to off-white powder	White powder
IDENTIFICATION (FT-IR): OP 200-227 v.8	Conforms to standard	Conforms to standard
^{1}H NMR: OP 200-221 v.17	Conforms to standard	Conforms to standard
IDENTIFICATION (HPLC): OP-018724 v.3	Retention time ratio within 0.98 to 1.02 of standard	Retention time ratio 1.00 of standard
ASSAY (HPLC): OP-018724 v.3	NLT 98.0%	99.9%
IMPURITIES (HPLC): OP-018724 v.3	No impurities detected greater than or equal to 1.0%	0.34% Rr 0.78 0.26% Rr 0.94 0.08% Rr 1.10 0.35% Rr 1.73
RESIDUAL SOLVENTS: OP 200-992 v.6	Methanol: NMT 3000ppm Dichloromethane: NMT 600ppm Ethanol: NMT 5000ppm 2-Methyl-tetrahydrofuran: NMT 5000ppm Isopropyl alcohol: NMT 5000ppm Tetrahydrofuran: NMT 720ppm Heptane: NMT 5000ppm Ethyl Acetate: NMT 5000ppm	<10ppm None detected None detected None detected None detected None detected 10ppm None detected
WATER CONTENT: OP 200-232 v.10	Report	0.1%
RESIDUE ON IGNITION: OP 200-248 v.5; <USP 281>	NMT 0.1%	< 0.1%
DSC: OP 100-232 v.2	Report Onset	175.1 °C
HEAVY METALS (ICP-MS): Exova Laboratories	Palladium: NMT 50ppm NMT 10ppm total USP metals	Palladium: <0.1ppm; LOD = 0.006ppm[1] NMT 10ppm USP metals
MICROBIAL LIMITS: <USP 61>	Total aerobic microbial count: NMT 100 CFU/g	<50 CFU/g

SIGMA-ALDRICH®

SAFC Pharma™
Inspiring Science

645 Science Drive, Madison, WI 53711 USA
Tel: (608) 233-3115 Fax: (608) 233-6873
www.safcglobal.com

BACTERIAL ENDOTOXIN: <USP 85>	NMT 10 EU/mg	<5.0 EU/mg

[1]LOD = limit of detection

This product was manufactured in compliance with current FDA Good Manufacturing Practices Regulations by SAFC, Inc.
Manufacturing Site: SAFC, Inc., 645 Science Dr., Madison, WI, 53711.

Tammy Herman
Quality Control Supervisor
November 29, 2010

Appendix III. Certificates of Analysis: 31-Day Stability of Product Solution

University of California, Davis
University of California Davis Medical Center
4635 2nd Avenue, Research I, Suite 1204
Sacramento, California 95817, USA

CERTIFICATE OF ANALYSIS

MATERIAL:	Product L (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 6% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 0.500 mg/mL)
LOT NO.:	1
MANUFACTURE DATE:	July 20, 2012
APPEARANCE:	Clear solution
ASSAY:	0.485 ± 0.003 mg/mL
IMPURITY LEVELS:	No peaks were seen that were not present in 0.9% sodium chloride, USP
pH:	5.447 ± 0.025
OSMOLALITY:	412.3 ± 3.51 mmol/kg
PARTICULATES:	No visible particles at 400x magnification
31-DAY STABILITY	Pass (+3.12% pH, +2.59% osmolality, and +4.92% concentration after 31 day storage at 4 °C)

Values are reported as mean ± standard deviation of triplicate measurements.

This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817

Gerhard Bauer
Laboratory Director GMP Facility
Adjunct Assistant Professor
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Chun-Yi Wu, Ph.D.
Analytical Chemist
Department of Neurology
University of California, Davis

August 22, 2012

University of California, Davis
University of California Davis Medical Center
4635 2nd Avenue, Research I, Suite 1204
Sacramento, California 95817, USA

CERTIFICATE OF ANALYSIS

MATERIAL:	Product H (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 6% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 1.500 mg/mL)
LOT NO.:	1
MANUFACTURE DATE:	July 20, 2012
APPEARANCE:	Clear solution
ASSAY:	1.664 ± 0.003 mg/mL
IMPURITY LEVELS:	No peaks were seen that were not present in 0.9% sodium chloride, USP
pH:	5.437 ± 0.042
OSMOLALITY:	412.3 ± 1.53 mmol/kg
PARTICULATES:	No visible particles at 400x magnification
31-DAY STABILITY	Pass (-2.51% pH, +1.70% osmolality, and +0.88% concentration after 31 day storage at 4 °C)

Values are reported as mean ± standard deviation of triplicate measurements.

This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817

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University of California, Davis

August 22, 2012

Appendix IV. Certificates of Analysis: 56-Day Stability of Concentrate

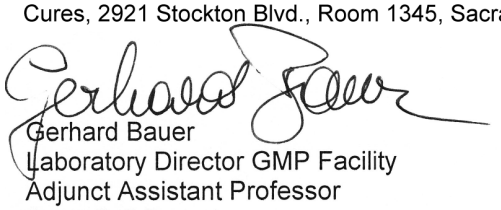
University of California, Davis
University of California Davis Medical Center
4635 2nd Avenue, Research I, Suite 1204
Sacramento, California 95817, USA

CERTIFICATE OF ANALYSIS

MATERIAL:	Product L, 4X concentrate (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 24% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 2.000 mg/mL)
LOT NO.:	2
MANUFACTURE DATE:	August 24, 2012
APPEARANCE:	Clear solution
ASSAY:	0.551 ± 0.001 mg/mL, 1X final product, diluted from the 4X concentrate with 0.9% sodium chloride, USP, on October 19, 2012
IMPURITY LEVELS:	No peaks were seen that were not present in 0.9% sodium chloride, USP
pH:	5.007 ± 0.058
OSMOLALITY:	408.0 ± 3.00 mmol/kg
PARTICULATES:	Pass [USP<788>, Light Obscuration Method (Method 1, test 1.A.), performed at Nelson Laboratories, Salt Lake City, UT], >10 μm particles: 1 particle/ml; >25 μm particles: 0 particles/ml.

The 4X concentrate was stored at –20 °C for 56 days.
Values are reported as mean ± standard deviation of triplicate measurements.

This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817



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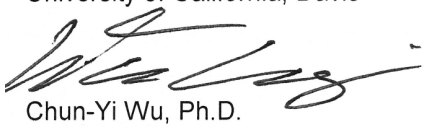

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October 19, 2012

University of California, Davis
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Sacramento, California 95817, USA

CERTIFICATE OF ANALYSIS

MATERIAL:	Product H, 4X concentrate (Allopregnanolone Intravenous Solution in 0.9% sodium chloride, USP with 24% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts, 6.000 mg/mL)
LOT NO.:	2
MANUFACTURE DATE:	August 24, 2012
APPEARANCE:	Clear solution
ASSAY:	1.530 ± 0.008 mg/mL, 1X final product, diluted from the 4X concentrate with 0.9% sodium chloride, USP, on October 19, 2012
IMPURITY LEVELS:	No peaks were seen that were not present in 0.9% sodium chloride, USP
pH:	5.047 ± 0.015
OSMOLALITY:	409.3 ± 1.53 mmol/kg
PARTICULATES:	Pass [USP<788>, Light Obscuration Method (Method 1, test 1.A.), performed at Nelson Laboratories, Salt Lake City, UT], >10 μm particles: 2 particles/ml; >25 μm particles: 1 particle/ml.

The 4X concentrate was stored at –20 °C for 56 days.
Values are reported as mean ± standard deviation of triplicate measurements.

This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817

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October 19, 2012

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CERTIFICATE OF ANALYSIS

MATERIAL:	Product Placebo, 4X concentrate (0.9% sodium chloride, USP with 24% β-cyclodextrin Sulfobutyl Ethers, Sodium Salts)
LOT NO.:	2
MANUFACTURE DATE:	August 24, 2012
APPEARANCE:	Clear solution
ASSAY:	ND mg/mL, 1X final product, diluted from the 4X concentrate with 0.9% sodium chloride, USP, on October 19, 2012
IMPURITY LEVELS:	No peaks were seen that were not present in 0.9% sodium chloride, USP
pH:	4.913 ± 0.058
OSMOLALITY:	420.0 ± 1.00 mmol/kg
PARTICULATES:	Pass [USP<788>, Light Obscuration Method (Method 1, test 1.A.), performed at Nelson Laboratories, Salt Lake City, UT], >10 μm particles: 0 particles/ml; >25 μm particles: 0 particles/ml.

The 4X concentrate was stored at –20 °C for 56 days.
Values are reported as mean ± standard deviation of triplicate measurements.

This product was manufactured in compliance with current FDA Good Manufacturing Practice Regulations by the University of California, Davis Good Manufacturing Practices Laboratory, Institute For Regenerative Cures, 2921 Stockton Blvd., Room 1345, Sacramento, CA 95817

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October 19, 2012

Appendix V. Product Label

I.V. Bag #:_____ Subject #: _____

Allopregnanolone or Placebo I.V. Solution in 0.9% sodium chloride injection, USP with 6% sulfobutyl ethers β-cyclodextrin sodium salts

BAG SEQUENCE # ___ of ___		
☐ Load	☐ Maintenance	☐ Taper

This container contains _____ mL

To Be Completed by Pharmacy:

Administer at _____ mL/min

Expiration Date: _____/____/_____

Protocol Name: IV Allopregnanolone Treatment of Patients with Acute Traumatic Brain Injury.
UC Davis IRB Protocol # 27314; IND 111,085.
Store at 2–8 ºC; dispense at room temperature.
For intravenous use only.
Caution: New Drug – Limited by Federal Law to Investigational Use
Manufactured by University of California, Davis